DIRECT EFFECTS OF INCREASING TEMPERATURE ON BIRDS

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

(Signature) ……

THESIS SUMMARY

Increasing air and sea temperatures are driving global ecological change. Here, I explore the direct effects of increasing temperatures on birds, which has thus far been studied less than the indirect effects. In **Chapter 1**, I review the literature to examine adaptations that enable birds to maintain core body temperatures in response to increasing heat loads, before summarising the effects that exposure to high temperatures can have. I also discuss the avenues for potential research in this field and highlight the importance of incorporating direct effects of increasing temperatures into future prediction models. In **Chapter 2** I investigate the impact of increasing temperatures on two closely related pelagic birds, constrained to come to land to breed in areas with differing temperature regimes. Specifically I quantify the proportion of time that northern gannets *Morus bassanus* (breeding in the boreal north Atlantic) and cape gannets *Morus capensis* (breeding in the Mediterranean biome of South Africa) spend gular fluttering (a proxy for heat stress), and examine the consequence of this for evaporative stress. I found that whilst gular fluttering did not correlate with humidity, it did increase asymptotically with ambient temperature. However, the slope and intercept vary between different age categories and across species, with the onset of gular fluttering occurring at lower ambient temperatures for younger birds and northern gannets in general. I also find that evaporative water loss increases with gular fluttering for all ages and across species, with higher rates of water loss found for the heavier northern gannets. Our predictive models show that rates of both gular fluttering and evaporative water loss will increase based upon climate change projections and subsequently we can conclude that by 2100 increasing temperatures will

have seriously impacted both northern and cape gannets, particularly birds constrained to the nest. Impacts are likely to be more severe for the northern gannets due to the greater change in ambient temperatures predicted at higher latitudes and the bird's lower gular fluttering onset thresholds and high rates of evaporative water loss. The findings presented here support the theory that increasing temperatures pose a serious and direct threat to birds and that the associated negative impacts may occur more rapidly than indirect effects. They also highlight the need for greater research into spatial and temporal patterns of heat stress, both within and between species. Finally, we propose the incorporation of the direct effects of increasing temperatures into future prediction models.

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AUTHORS DECLARATION

Unless stated below, Calum E. Laver carried out all literature searches, data processing, data analysis, manuscript writing, thesis compilation and formatting. Dr Stephen Votier, Dr Richard Sherley, Dr Timothée Cook and Dr Ilya Maclean provided guidance and comments throughout the project. Dr Andrew Bladon and Dr Paul Donald provided additional comments and guidance for Chapter 1. The project was designed based upon work by Greg Campbell and Pauline Dufour under the supervision of Dr Timothée Cook and Dr Richard Sherley. The work of Greg Campbell and Pauline Dufour was provided under permission and used to help inform the methodology and provide a structure for the research project to follow.

On Grassholm, Calum E. Laver collected all video and climate data under the supervision of Dr Stephen Votier with permission from the Royal Society for the Protection of Birds (RSPB). On Malgas, Calum E. Laver collected all video and climate data under the supervision of Dr Timothée Cook with permission granted by South African National Parks Service (SANParks). All data collection was remote and non-invasive.

A team of undergraduate volunteers assisted with processing of the video data and analysis of the thermoregulatory behaviours.

GENERAL INTRODUCTION

Birds are globally distributed and are found in a diverse range of environments across all seven continents (Sibley & Monroe, 1990). They are easy to observe and for many provide enjoyment and a sense of connection with the natural world (Jones, 2011). As a result they are exceptionally well studied are amongst the best monitored organisms on the planet thanks to the vast amounts of data continuously collected by scientists, amateur ornithologists and the general public (Pearce-Higgins & Green, 2014). One key reason for the geographic distribution and success of birds is that they are endotherms, capable of maintaining their body temperatures in response to variation in environmental temperatures through a diverse range of physiological and behavioural adaptations (Dawson & O'Connor, 1996). This has enabled them to survive in some of the harshest conditions on the planet including polar and desert regions, with species more likely to be constrained by the energetic requirements of thermoregulation than by thermal tolerance (Buckley, Hurlbert & Jetz, 2012).

Despite their popularity and extensive scientific research, many species of birds are under threat. 13% of the 10,064 bird species are considered threatened by extinction within the next 100 years and another 880 species have been identified as near threatened (Pearce-Higgins & Green, 2014). Furthermore, current rates of population decline do not appear to be slowing, whilst indicators of the pressures on biodiversity show continued increases (Butchart *et al.* 2010). Degradation and loss of suitable habitat, invasive species and overexploitation all pose a significant risk to birds at an individual and

population level. Alongside these, anthropogenic climate change presents an increasingly significant challenge and is likely to become an issue of even greater importance throughout the course of this century (Warren *et al.* 2013). Consequently, understanding the current effects and future impacts of climate change is a critical area of research, with globally important consequences.

For birds, perhaps the most prevalent impacts of climate change are the resultant increases in air and sea surface temperature. These increases are occurring at an unprecedented rate, significantly faster than previously predicted based upon natural fluctuations (Pachauri *et al*. 2014) and are forecast to continue increasing (Solomon *et al.* 2007). Changes in temperature have the potential to alter species fitness (Boyles *et al.* 2011) and are an important determinant of distribution, demography, phenology, physiology and behaviour (Walther *et al.* 2002; Parmesan, 2006; Pearce-Higgins & Green, 2014). The effects of changing temperatures can be broadly categorised into two types. The first are direct effects. These are the direct processes through which temperature changes affect the environment, growth and development rates and behaviour of birds (Ockenden *et al.* 2014). The second are indirect effects. These are the mechanisms where by the effects of increasing temperature on an individual or population are interceded via effects on another species, such as altered phonological interactions, changes in predator-prey dynamics, and changes in resource availability (Ockenden *et al.* 2014). Avian responses to both the direct and indirect effects have already been observed across numerous species however the impacts are likely to vary between species due to differences in life history and ecology (Oswald and Arnold, 2012)

and within species – a result of individual differences in exposure to ambient temperatures and extreme weather (Newell *et al.* 2015).

The indirect effects of increasing temperatures on birds are often considered to be more important than the direct effects (see Ockenden *et al.* 2014). This is due to the inherent endothermy of birds, allowing them to buffer against temperature variation of their surrounding environment or changes in radiative heat loads, through physiological adaptations or active thermoregulation. However, active thermoregulation requires an increase in energetic expenditure, reducing the amount of energy that is available for investment in other critical functions such as foraging, reproduction or growth (Boyles *et al.* 2011). Furthermore direct effects are likely to have a much faster and more immediate effect than those posed by indirect effects such as changes in the availability of food resources, which are likely to be delayed in comparison.

Birds have been shown to be extremely sensitive to the effects of increasing temperatures; among 110 common birds breeding across Europe, species less tolerant to warm climates have shown the sharpest declines between 1980 and 2005 (Jiguet *et al.* 2010). Despite this, the direct effects of increasing temperature have received less attention than the indirect effects and are rarely considered in survival models.

This research

To address the lack of a synthesis of the direct effects of increasing temperature on birds I have produced a review of current literature,

summarising the major methods used by birds for thermoregulation, highlighting current knowledge gaps and identifying potential avenues for future research. I then build upon this by investigating the direct effect of increasing temperatures on two closely related pelagic birds using remote monitoring methods. In combination with environmental data, the remote footage allows for analysis of the variation in behavioural response of these two species to the direct effects of increasing temperatures, across multiple age categories as well as calculations of the costs of these responses to be made. The incorporation of temperature data, based upon current climate change projections, allows for the future impacts of increasing temperatures on these two species to be understood.

CHAPTER 1 – A REVIEW OF THE DIRECT EFFECT OF INCREASING TEMPERATURES ON BIRDS

Abstract

Climate change represents a significant challenge to avian biodiversity, but the majority of research has focused on indirect effects. Direct effects include the physiological stress of extreme high temperatures, which are predicted to increase as climates warm. Here we review adaptations of birds to extreme high temperatures, illustrating the evolutionary importance of thermoregulation its cost at high temperatures (heat stress) and importance of water loss (evaporative stress). We then review the direct effects of increasing temperatures on birds at different life-history stages. We conclude that both heat and evaporative stress have profound consequences for avian fitness, impacting behaviour, reproductive success and adult survival, with eventual impacts on demography and distributions. Furthermore, these are likely to have a more immediate impact than indirect effects, which are often lagged. Future studies will need to redress this bias and, in particular, should focus on disentangling the direct and indirect mechanisms that underpin the impacts of climate change on birds.

Introduction

Avian biodiversity is under threat from habitat loss (Brooks *et al.* 2002), introduced species (Vitousek *et al.* 1997), overexploitation (Groom, Meffe & Carroll, 2006) and anthropogenic climate change (Thomas *et al*. 2004). Increased atmospheric greenhouse gases (Solomon *et al*. 2007) are linked with unprecedented rates of temperature increase (Jones *et al*. 2007, Pachauri *et al*. 2014), with average global land and ocean surface temperature predicted to rise by as much as 2–5°C by 2100 relative to pre-industrial levels (IPCC, 2014, Brown & Calderia, 2017) (Figure 1). The International Union for the Conservation of Nature (IUCN) considers 35% of bird species assessed to be susceptible to climate change impacts (Foden *et al.* 2008). To date most climate change research on birds has focused on indirect effects, the mechanisms where by the effects of increasing temperature on an individual or population are interceded via effects on another species (Ockenden *et al.* 2014). These include alterations in food quality and quantity (Cohen *et al.* 2014), interspecific and intraspecific interactions (Parmesan, 2006, Tylianakis *et al.* 2008;) and habitat change (Galbraith *et al.* 2002). However, the direct effects of increasing temperatures on birds, the direct processes through which temperature changes affect the environment, growth and development rates and behaviour of birds (Ockenden *et al.* 2014)., such as altered thermoregulatory ability (du Plessis *et al.* 2012) and water balance (Albright *et al.* 2017), have received much less attention (but see Oswald & Arnold, 2012). This is possibly because thermal homeostasis is disconnected from environmental conditions in endotherms, so the indirect effects are considered to be of greater importance (Ockenden *et al.* 2014).

Despite receiving less research than indirect effects, the direct effects of high temperatures may have profound consequences for birds (Boyles *et al.* 2011; Oswald & Arnold, 2012; Wolf *et al.* 2016). In endotherms, heat produced metabolically must be dissipated to avoid body temperatures reaching lethal limits (Figure 2). Additionally, birds may absorb heat from the environment, which they must also shed in order to maintain thermal homeostasis. Thus, maintaining a stable body temperature in changing ambient conditions requires the allocation of time and energy to active thermoregulation instead of growth or reproduction (McNab, 2002, 2012).

This shift in resource allocation is known as the 'biological cost of stress' (Moberg, 2000). The capacity to get rid of internal heat may limit maximal energy expenditure directly (the heat dissipation limit theory) and so constrain behaviour, leading to heat stress at high ambient temperatures (Speakman & Król 2010). The term heat stress is commonly used to mean the demand made by the environment for increased heat dissipation at high temperatures (Silanikove, 2000) and could be especially problematic for birds, relative to mammals, as they have high metabolic rates and are more commonly diurnal, features making life in hot environments difficult. Moreover, although birds are considered important bellwethers of climate change (Crick, 2004), research on the direct effects of increasing global temperatures lacks a synthesis. Here we provide a summary of the mechanisms through which birds mitigate heat stress, and then explore the ways in which they are affected by high temperatures and how this may be altered in a warming world. We finish by focusing on potential avenues for future research.

Avian thermoregulation: general adaptations for managing heat stress

What is heat stress? – Increased thermoregulatory demands at temperatures above an animal's thermoneutral zone can increase allocation of metabolic energy to maintain thermal homeostasis: this is heat stress (Silanikove, 2000) and can cause the denaturation of proteins and death (Ritchie *et al.* 1994). Although maintaining body temperature within the thermoneutral zone is equally important in terms of resource allocation when exposed to high and low temperatures, heat stress associated with high temperatures may be of greater concern in endotherms (birds and mammals) than cold stress associated with low temperatures because the margin between core temperature and lethal hyperthermia is far smaller than for lethal hypothermia (Blache *et al.* 2011). Thus, the ability to maintain body temperature when exposed to high temperatures is essential, especially for birds occupying desert and tropical environments.

There is considerable variation in the body temperatures of birds, a review by Prizinger et al. (1991) found mean levels of body temperature for birds of 38.54±0.96°C during periods of rest and elevated body temperatures of 43.85 ±0.94°C during periods of high activity such as flying or running with temperatures as high as 47.7°C recorded in the white-crowned sparrow *Zonotrichia leucophrys* (Southwick, 1973). Despite the considerable variation in avian body temperatures, managing heat loads and minimising prolonged periods of elevated body temperatures is critical for long term survival.

In birds, heat is dissipated through conduction, convection, radiation and evaporation (Figure 3) either behaviourally or physiologically (McNab, 2012). Behavioural strategies for managing heat stress include: postural adjustments that increase the body surface exposed to air (Ward *et al.* 2008), straddling of the nest to provide shading (Brown & Downs, 2003), increasing blood flow to the legs and feet (Bernstein, 1974), exposing thinly feathered areas to air flow (Howell & Bartholomew, 1961), bathing (Oswald *et al.* 2008), belly soaking (Amat & Masero, 2007), extending the wings away from the body (Cook & Leblanc, 2007) and excreting on to their own legs (Kahl, 1963). Cool thermal refugia can also be used to minimise the risk of heat stress and aid thermoregulation (Williams & Tieleman, 2001; Cunningham *et al.* 2015; Martin *et al.* 2015). For example, when temperatures rise above 31°C Magpies *Pica pica* will spend more time perched in the shade and less time foraging (Kelly *et al*. 2004) as do Ethiopian Bush-crows *Zavattariornis stresemanni* (Bladon, 2017). Desert birds show similar responses (Austin & Miller, 1982; Thompson *et al.* 2018); for example Cactus Wrens *Campylorhynchus brunneicapillus* select cooler microhabitats when ambient temperature rises above 35°C (Ricklefs & Hainsworth, 1968) and Northern Bobwhites *Colinus virginianus*, which avoid habitat patches warmer than 39°C (Forrester *et al.* 1998). Eventually, some birds undergo relatively long distance movements to escape temporary heat waves (‘heat nomadism’) (Davies, 1984; Dean, 2004). However, it is unclear how widespread this behaviour might be, or if it is efficient in view of the energetic consequences of travelling or abandoning a nest or territory. Nomadism in desert birds is common, but normally reflects an adaptation to ephemeral local food and water resources (Dean, 2004).

Physiological adaptations to heat are numerous. Constraints for heat dissipation shape avian morphology. Feathers facilitate loss of excess heat via ptiloerection (plumage compression and elevation; Bartholomew & Dawson, 1979), radiation, convection or conduction (Dawson, 1982) (Figure 3). Additionally, plumage colour and structure influence heat loads acquired from solar radiation; birds with darker plumage typically experience greater heat loads than those with lighter feathers (Wolf & Walsberg, 2000, Hochscheid *et al.* 2002). Across species, beak size (controlling for body mass) correlates positively with air temperature (Symonds & Tattersall 2010), in part because this highly vascularised structure plays an important role in thermoregulation by controlling blood flow in response to temperature (Symonds & Tattersall 2010, Tattersall 2016, Van de Ven *et al.* 2016). Adult Toco Toucans *Ramphastos toco*, for example, are able to dissipate heat via vasodilation inside the beak (Tattersall *et al.* 2009). Furthermore, the positive relationship between body size and latitude in birds, as predicted by Bergmann's rule (e.g. McCollin *et al.* 2015), as well as the shorter appendages of birds in cold environments, as predicted by Allen's rule (e.g. Symonds & Tattersall, 2010), are probably related to thermoregulation (although fasting endurance may also be important; Ashton 2002).

As air temperature rises towards body temperature, some birds may allow their body temperature to rise by up to 5°C (Nilsson *et al.* 2016). This 'adaptive hyperthermia' allows them to maintain a temperature gradient between the body and the air to facilitate dry heat transfer and reduce otherwise costly evaporative water loss (Tieleman & Williams, 1999; Nilsson *et al.* 2016). For example, White-browed Sparrow Weavers *Plocepasser mahali* living in desert

environments are able to continue foraging during the warmest part of the day by allowing their core body temperature to rise higher than that on conspecifics from cooled, semi-desert environments (Smit *et al.* 2013).

When ambient heat equals or exceeds body temperatures, the thermal gradient between a bird and its environment disappears or becomes positive. At this point, heat loss by radiation and convection cease to be useful and evaporative heat loss may be the only mechanism by which a bird can lose heat effectively, provided it retains access to water. Panting enables evaporative heat loss from the moist surfaces of the mouth, pharynx and throat. Most birds pant, but some supplement evaporation from the respiratory tract by fluttering the membranous gular area; this is known as gular fluttering (Bartholomew *et al.* 1968). This has been recorded in nightjars (Caprimulgiformes), roadrunners (Cuculiformes), mousebirds (Coliiformes), owls (Strigiformes), doves (Columbiformes), pelicans (Pelecaniformes), as well as cormorants (Pelicaniformes), gannets, anhingas, frigatebirds and boobies (all Suliformes) and many gallinaceous species (Bartholomew *et al*. 1968, Lasiewski, 1969, Dawson, 1982). However, the efficiency of panting and gular fluttering depends on the temperature and humidity of the surrounding air (Oswald & Arnold, 2012). Respiratory evaporation results in high evaporative water loss (e.g. Lasiewski, 1969, Hochscheid *et al.* 2002) and is typically used when other mechanisms for heat dissipation are not possible, for instance when adults are incubating or chick-rearing (Dawson, 1982).

Although birds lack sweat glands, cutaneous water loss represents 45−63% of total evaporative water losses in small birds (Bernstein, 1971). In Verdins

Auriparus flaviceps, water loss occurs almost evenly between respiratory and cutaneous components at moderate air temperatures, although respiratory evaporation is the primary form of heat dissipation (Wolf & Walsberg, 1996). Hence, the respiratory pathway is generally considered more efficient and several desert birds have developed the ability to curtail cutaneous water loss in this water poor environment by seeking relatively cool, shaded microsites at the hottest parts of the day in order to minimise heat load and cutaneous water loss (Dawson, 1982; Wolf & Walsberg, 1996). Columbids are a notable exception, as they are capable of dissipating essentially their entire heat load through cutaneous evaporation at high temperatures (Marder & Arieli, 1988). Thus, evaporative heat loss is an effective route by which many birds can maintain their body temperature far below ambient air temperatures, even during acute heat exposure. Three species of ploceid weaver tolerate experimental exposure to air temperatures from 48–54°C, with evaporative heat dissipation reaching 141–222% of metabolic heat production (Whitfield *et al.* 2015). Arid-zone columbids (Namaqua Dove *Oena capensis*, Laughing Dove *Spilopelia senegalensis*, Cape Turtle Dove *Streptopelia capicola* and Crested Pigeon *Ocyphaps lophotes*) maintain body temperatures below 45°C, when exposed experimentally to air temperatures up to 62°C, through evaporative heat loss between 227 and 446% of metabolic production (McKechnie *et al.* 2016).

The effect of heat stress on bird fitness

Despite their efficient behavioural, physiological and evolutionary strategies for managing heat loads, birds sometimes face difficulties in coping with high

temperatures (for a summary see Table 1 & Figure 4). Depending on the level or duration of heat, and/or bird activity, fitness may be negatively affected.

Effects on survival – In extreme circumstances, heat stress can lead directly to death (Warriss *et al.* 2005). The upper limit on body temperatures before lethal hyperthermia sets in is thought to be 46– 47°C in birds (Nilsson *et al*. 2016) and even most desert adapted species are unable to cope with prolonged exposure to ambient temperature of >45°C (Tieleman & Williams, 1999). This is because when the body's core exceeds this temperature threshold, proteins begin to denature and enzyme activity diminishes, leading to death (Daniel *et al.* 2010; Del Vesco *et al.* 2015). Mass mortality events during major heat waves, such as those occasionally reported in Australia, are striking examples of this. The most catastrophic on record occurred during 1932 in southern central Australia, when millions of birds of different species died of heat exposure when air temperatures remained around 50°C for several consecutive days. Direct hyperthermia (not dehydration *per se*) was suspected to be the main cause of death in many cases, with individual rates of evaporative heat loss unable to cope with excess heat load in birds (McKechnie *et al.* 2012). Aside from mortality related to extreme hyperthermia, chronic exposure to heat stress may affect adult immune function, reducing white blood cell counts and inhibiting antibody production, resulting in increased risk of illness, reduction in immune response and reduced survival (Mashaly *et al.* 2004).

Effects on foraging – In the Kalahari, when daily maximum air temperatures exceed 35.5°C, Southern Pied Babblers *Turdoides bicolor* trade-off foraging for behavioural thermoregulation leading to weight loss (du Plessis *et al.* 2012).

Similarly, Ethiopian Bush-crows *Zavattariornis stresemanni* increase panting behaviour and reduce food intake rates as temperatures increase (Bladon 2017).

Effects on adult survival during reproduction – For birds with bi-parental care, being temporarily constrained to the nest can prolong heat stress and increase energy expenditure or mortality risk. For example, heat stress during moult and breeding in Little Penguins *Eudyptula minor* negatively impacts adult survival (Ganendran *et al.* 2016), while for Ruddy Turnstones *Arenaria interpres*, measurements of daily energy expenditure using the doubly-labelled-water technique showed that, during the incubation and brooding period, thermoregulation can account for up to 31% of an individuals daily energy expenditure whilst 22% could be attributed to basal metabolic rate and 44% to the cost of activity (Piersma & Morrison, 1994).

Effects on breeding success - To minimise any negative impacts of heat stress during incubation and chick-rearing, adults may need to increase nest absences for thermoregulation, increasing risk of predation and thermal exposure of the nest contents (Gaston, Hipfner & Campbell, 2002). Under high thermal loads, incubating penguins and cormorants will abandon breeding in favour of individual survival (Boersma, 1975; Sherley *et al.* 2012a,b)., while ground-nesting shorebirds select sites with minimal shade to provide cover from solar radiation in order to allow increased vigilance and reduce predation risk. However, heat stress can force these incubating birds to leave the nest in order to bathe, increasing the likelihood nest predation (Amat & Masero, 2004). A similar thermoregulation-predation trade-off has been reported in Great Skuas

Stercorarius skua (Oswald *et al.* 2008), Northern Mockingbirds *Mimus polyglottos* (Londono, Levey & Robinson, 2008) and Hoopoe Larks *Alaemon alaudipes*; this last species incubates in open areas, increasing vigilance for predators, but shifts nests to the shade of bushes as the breeding season progresses, reducing thermoregulatory costs but increasing nest predation rates (Tieleman *et al.* 2008). Breeding birds clearly face an important trade-off between self-care and parental care (Salzman, 1982; Brown & Downs, 2003; Londono, Levey & Robinson, 2008), with species that perform long foraging trips likely at greater risk of heat stress when nesting than congeners that undertake shorter trips (Oswald *et al*. 2011).

Effects on egg and chick viability and survival – The impacts of heat stress are likely to be greatest for eggs and chicks (Whittow & Tazawa, 1991). Whilst developing embryos can have broad thermal tolerances (Webb, 1987), incubation temperatures outside optimal limits can impact individual growth and immune response, lowering fitness (Ardia *et al.* 2010). Embryos are vulnerable to hyperthermia (Webb, 1987) and consequently adult birds in hot environments must spend a greater proportion of time cooling their eggs (Londono, Levey & Robinson, 2008). During early development, chicks have a limited thermoregulatory ability compared to adults (Ardia *et al.* 2010), putting them at greater risk of negative impacts on growth and survival (Redpath *et al.* 2002; Greño *et al.* 2008).

Parents can respond to this by building nests in more thermally stable environments (Wakelin, Wilson & Downs, 2013). However, this is not always possible and eggs or chicks incubated or raised at high temperatures may

produce fledglings with diminished fitness. Nestling thermoregulatory capacity develops quicker in species where adults make longer foraging trips (e.g. hirundines), as there is greater pressure on chicks to resist changes in environmental temperatures when the parents are away (O'Connor, 1975). Once this capacity has developed, warmer nest environments tend to be beneficial to chick growth and survival (Reid *et al.* 2000; Pérez *et al.* 2008), up to a point. For instance, Spotless Starling chicks *Sturnus unicolor* have lower mass and smaller tarsi at higher ambient temperatures (Salaberria *et al.* 2014). Moreover, in Common Fiscals *Lanius collaris,* the frequency with which daily maximum air temperatures exceeded critical thresholds reduced fledgling body mass at a critical threshold of 33°C, tarsus length at 35°C and delayed timing of fledging at 37°C (Cunningham *et al.* 2013a). Eventually, birds raised at high temperatures can suffer severe physiological changes later in life, including decreased food intake, reduced feeding efficiency, declines in egg production and lower egg quality (El-Tarabany, 2016).

In extreme cases, nest survival itself may be threatened. As air temperatures exceed 35°C in the north American prairies, nest survival of Horned Larks *Eremophila alpestris*, McCown's Longspur *Rhynchophanes mccownii*, Chestnut-collared Longspur *Calcarius ornatus*, Lark Bunting *Calamospiza melanocorys* and Western Meadowlark *Sturnella neglecta* all decrease, suggesting that eggs and chicks are vulnerable to such temperatures (Conrey *et al.* 2016). Direct chick mortality associated with high ambient temperatures has also been noted in some seabirds, including Bank Cormorants *Phalacrocorax neglectus* (Sherley *et al.* 2012b) and Glaucous-winged Gulls *Larus glaucescens* (Gillet, Hayward & Stout, 1975).

The effect of evaporative stress on bird fitness

Many fatal impacts of heat are related to the associated dehydration. An understanding of evaporative stress is therefore crucial to appreciate the overall impacts of heat stress, although it may not always be possible to disentangle its effects from that of direct hyperthermia.

What is evaporative stress? – Evaporative stress can be defined as the use of evaporative cooling to avoid hyperthermia in response to high temperatures and typically results in high levels of water loss. Evaporative water loss is the only effective method for heat dissipation once the air temperature has increased above the bird's core temperature i.e. ~ 40°C (Figure 2, Figure 4). Respiratory evaporation behaviour such as panting and gular fluttering are costly in terms of water loss and can lead to severe dehydration in the absence of an adequate water supply (Wolf & Walsberg 1996). Furthermore, panting involves heavy breathing, which requires active muscle movements, and thus important energy expenditure (Smith *et al.* 2015, Fig. 2). The muscles driving the hyoid during gular fluttering, however, are small compared to the metabolically active mass of a bird; hence, the energy required for this behaviour is presumably low (for a review, see Lasiewski & Snyder 1969). Cutaneous evaporation, an important route for evaporative cooling in columbids, is also considered to be energetically undemanding (Smith *et al.* 2015).

Evaporative cooling, water balance and survival - Beyond the problem of access to water, the inability to evaporate water at a fast enough rate to balance heat loads may result in a lethally elevated body temperature (McKechnie & Wolf, 2010; Albright *et al*. 2017). As seen above in the example of birds exposed to heat waves in Australia, birds can die from hyperthermia, even when sufficient water is available, simply through their inability to dissipate heat fast enough (McKechnie & Wolf, 2010). In another example, Towhees *Pipilo. spp* become hyperthermic at body temperatures above 40 °C, lose the ability to carry out coordinated movements when this reaches 45 °C and die at 47 °C (Dawson, 1954). Rates of evaporative water loss are dependent on body mass; smaller species face a greater risk of dehydration than larger ones due to their higher mass-specific rates of evaporative water loss (Albright *et al.* 2017) , but larger species are less likely to dissipate heat fast enough as a result of their lower surface area-volume ratios and increasing thermal inertia associated with increasing body mass (Smit *et al.* 2016). Moreover, when water loss via thermoregulation exceeds water intake, birds may face a trade-off between thermoregulation, hydration and activity (Smit & McKechnie, 2015), which is particularly problematic in the face of increased frequency and duration of hot weather events (Cunningham *et al.* 2013b).

Effects of adults during reproduction - When birds are constrained by reproduction, increasing evaporative heat loss is likely to be one of the most effective strategies to thermoregulate. Thus, body water may become limiting. In marine birds, whose primary source of water is via ingested food, prolonged gular fluttering can lead to severe dehydration (Hochscheid *et al.* 2002). In Cape Gannets *Morus capensis*, the theoretical rate of evaporative water loss in

individuals with an elevated body temperature is 100% of daily-ingested water after 7.5 hours of continuous gular fluttering (Hochscheid *et al.* 2002). Even when inactive and completely shaded, the rate of water loss experienced by small birds is approximately 5% of body mass per hour (Wolf & Walsberg, 1996), leading to rapidly exceeding dehydration tolerances of approximately 22% body mass and resulting in severe dehydration and increased likelihood of mortality (McKechnie & Wolf, 2010). Extreme heat may also interact with other perturbations; in Arctic Canada the combination of high temperatures and abundant mosquitoes led to high mortality of adult Brünnich's Guillemots *Uria lomvia* (Gaston, Hipfner & Campbell, 2002). Here a combination of high temperatures, leading to water loss via panting to increase evaporative cooling, and mosquito attacks (and resultant blood loss) ultimately led to adult mortality via dehydration and heat stroke.

Effects on eggs and chicks – Eggs loose some proportion of water naturally through the porous shell. However, high ambient temperatures or low relative humidity may increase egg water loss and embryonic mortality (Davis *et al.* 1988; van der Pol *et al.* 2013). Dehydration may also be a particular problem for nestlings, as they are dependent on their parents for all of their water (usually via their food). For instance, Spotless Starling chicks exposed to prolonged periods of high heat experience dehydration, which negatively affects nestling fitness and survival (Salaberria *et al.* 2014). Although the effects of dehydration in chicks has received less attention than in adults, chicks are likely vulnerable to evaporative stress, if only because smaller birds experience higher rates of evaporative water loss than larger ones (Albright *et al.* 2017).

Long-term impacts of heat stress and water loss

Demographic effects - Species less tolerant to warm climates have shown the greatest population declines between 1980 and 2005 (Jiguet *et al.* 2010) and rising temperatures will likely result in increased heat stress. This will not only impact negatively on survival in the tropics and arid regions (where many birds already experience temperatures outside their thermal neutral zone), but also across temperate and polar regions (McKechnie, Hockey & Wolf, 2012; Oswald & Arnold, 2012; Khaliq *et al.* 2014). Rising temperatures will also increase evaporative water requirements, with probable impacts on survival (McKechnie & Wolf, 2010; Albright *et al*. 2017). Regional warming with consistent water availability or regional drying with consistent temperatures could therefore both lead to species being unable to survive in currently habitable areas. By the 2080s, desert bird survival in mid-summer is predicted to be significantly lower than at present, with more frequent nesting failures and catastrophic mortality events (McKechnie & Wolf, 2010). Indeed, extreme heat waves are forecasted to continue to increase in frequency and intensity in the near future (IPCC, 2014). These heat waves are likely to be a limiting factor affecting species persistence in a region, either via lethal, or cumulative sub-lethal, effects (Cunningham *et al.* 2013b).

Effects on distribution – Evidence for limitation in species' ranges due to high temperatures in endotherms is rare (Thomas, 2010) despite the clear existence of physiological limits to high temperatures and behaviours to avoid high temperatures. Nonetheless, by combining observed physiological or behavioural thresholds with range limits there is some evidence that species'

distributions are directly limited by climatic factors. For example, despite the presence of abundant prey and suitable breeding habitat at lower latitudes, Great Skuas *Catharacta skua* are confined to breed at high latitudes where summer temperatures are below 13°C (Furness, 1988). When temperatures exceed 14°C, Great Skuas increase bathing frequency, suggesting that their distribution may be determined by a physiological intolerance of heat (Oswald *et al.* 2008).

In the tropics, the Ethiopian Bush-crow *Zavattariorni stresemanni* is restricted to a small area of unremarkable acacia scrub, with seemingly suitable habitat and adequate food resources in the surrounding area (Donald *et al.* 2012; Jones *et al.* 2018). However, its distribution is almost perfectly described by the limits of a climate envelope where temperatures rarely exceed 30°C and, even at temperatures below this, Bush-crows exhibit strong behavioural responses (increased use of shade and panting) and restrictions (reduced food intake) when temperatures rise (Bladon, 2017). This is strongly suggestive of a direct effect of high temperature on the species' distribution.

Changes in species' distributions also highlight how high temperatures can limit geographical range. Over the last 25 years Pied Crows *Corvus albus* have shifted south-westwards across South Africa; the areas of highest abundance track a mean annual temperature of 19°C and population density has declined sharply in areas warmer than this (Cunningham *et al.* 2016). This response suggests that the Pied Crow's temperature preference could reflect a fundamental aspect of its physiology.

However, care must be taken to not automatically attribute the direct effects of increasing temperatures as the cause for correlations between temperature change and changes in species' abundance or distribution, unless mechanistic links have been clearly delineated. This is because even if temperatures define a potentially suitable area for a species, other intrinsic factors such as habitat suitability, competition, dispersal and predator-prey interactions often influence realised species ranges (Pearce-Higgins & Green, 2014; Milne *et al.* 2015). Since the 1980's, endemic Fynbos species occupying the coolest regions of South Africa have experienced the greatest reduction in population size and range, with the species whose ranges cover areas where air temperature has increased the most having suffered the greatest declines. However, only one of the 12 species studied, the Cape Rockjumper *Chaetops frenatus*, exhibited signs of a low physiological thermal threshold, namely the lowest air temperature threshold at which evaporative water loss started to increase (Milne *et al.* 2015).

As air temperatures continue to increase, it becomes more likely that physiological intolerances will become a more prevalent cause of extinction (Cahill *et al.* 2012). Birds may be better able to escape physiological impacts of increasing temperatures than many mammals, due to their highly mobile nature, and their distributions may more likely be driven by the ecological consequences of climate (Weathers & van Riper, 1982). However, thermoregulatory differences between similar species inhabiting different environments (Weathers & van Riper, 1982; Frumkin *et al.* 1986), and the climatically adaptive basal metabolic rates and heat loss strategies of many

species (Smit *et al.* 2013; Whitfield *et al.* 2015; McKechnie *et al.* 2016), suggest that physiological constraints can be important in determining bird species distributions.

Evolutionary effects - Morphological and physiological adaptations to conserve heat at low temperatures may impair the ability of birds to thermoregulate under predicted future climatic conditions (Nudds & Oswald, 2007; Oswald *et al.* 2008). Many species have not had time to adapt their behaviours in response to increasing temperatures and therefore mortality rates are predicted to rise (Carr & Lima, 2012; Boersma & Rebstock, 2014). Bergmann's rule has been hypothesised to be the principle behind the decrease in body size observed in several taxa of birds and mammals over the past few decades (e.g. Yom-Tov *et al.* 2006, Gardner *et al.* 2011). However, it is unclear whether this trend is an adaptation to increasing temperatures following natural selection or some form of phenotypic plasticity (Teplitsky *et al.* 2008).

Avenues for future research

Future research should aim to refine knowledge of avian thermoregulation, building on taxon-specific research, much of which is over half a century old (see Oswald & Arnold, 2012). Field research should endeavour to quantify the variation of direct effects within and among species with detailed, long-term studies on fitness and survival, identifying key annual or life-history periods of thermoregulatory constraint. By studying the same species, or closely related species, across multiple locations it may be possible to identify patterns of thermal adaptation within species or genera. This local adaptation has already

been demonstrated in the lab (Williams & Tieleman, 2000) and in the field (Smit *et al.* 2013), but studies remain scarce. These studies could make use of recently developed, non-invasive and affordable remote sensing technologies such as digital cameras, which can monitor thermoregulatory behaviour (e.g. postural adjustments or panting), or infrared thermography, which is a non-contact method for measuring bird surface temperature and temperature of the surrounding environment (McCafferty, 2013; Briscoe *et al.* 2014; Tattersall, 2016). More invasive field methods, like surgically implanted miniaturised electronic temperature recorders, can record fine scale body core temperature patterns that may be put in perspective with behaviour of birds that have been released, as observed visually or recorded on camera in the field (Smit *et al.* 2013). The development of gastrointestinal loggers, present a less invasive alternative to surgically implanted loggers (McCafferty *et al.* 2015). These allow a relatively non-invasive method of measuring internal temperature, recording temperature during passage through the gastrointestinal tract and have also been used in conjunction with other temperature loggers to examine physiological processes such as hypothermia (Handrich *et al.* 1997).

In parallel, laboratory (or field-laboratory) work on captive birds, although not necessarily mimicking the full range of environmental conditions to which wild birds can be exposed, can complement field data. This experimental approach remains important for understanding the physiological processes involved, and for measuring parameters such as heat tolerance limits, metabolic rates or evaporative water loss, including cutaneous losses (McKechnie *et al.* 2017). It is also likely to be crucial for confirming the mechanistic drivers of behaviour and separating the often confounded direct and indirect effects of climate

change. Undertaking experiments to understand whether heat dissipation efforts in birds are actually indicative of dehydration risk will also be important (Smit *et al.* 2016), as will studying the effect of humidity on bird evaporative cooling efficiency (Gerson *et al.* 2014). Humidity reduces the bird-to-air gradient driving evaporative heat loss, thus limits heat dissipation. However, although it is forecasted to increase with global warming, few studies have investigated humidity in the context of avian thermoregulation. Another possible avenue of experimental research concerns the study of the effect of temperature on chick limb growth. Warmer temperatures induce the growth of longer limbs in mice, something that may represent a form of phenotypic plasticity in accordance with Allen's rule (Serrat *et al.* 2008). To our knowledge, such experiments have not been performed on birds, although they may hold the key to understanding the role of phenotypic plasticity as a form of adaptation to increasing temperatures (Teplitsky *et al.* 2008).

Future research should also incorporate modelling to identify both species at greatest risk from and those most informative of the effects of increasing temperatures using the framework laid out by McKechnie, Hockey and Wolf (2012). This involves the incorporation of temperature dependence data for behavioural, physiological and reproductive variables into models of survival and reproduction under future climatic scenarios. From these models, more accurate predictions about the effects of climate change on individual species, including the capacity for physiological adaptation and the potential for range shifts or population declines, can be made (Kearney & Porter, 2009). These will facilitate the development of more generalised models for entire avian communities (McKechnie, Hockey & Wolf, 2012), highlighting the vulnerabilities

of species to increasing temperatures and providing a tool for predicting variation in species resilience.

Groups of birds particularly threatened by the direct effects of increasing temperatures

Birds that occupy marine, tropical, desert and urban environments appear to be particularly vulnerable to heat stress. Marine birds must balance the conflicting thermoregulatory constraints of nesting on land and foraging at sea (Oswald & Arnold, 2012; Sydeman, Thompson, & Kitaysky, 2012; Franklin *et al.* 2014). The widely espoused value of marine birds as biological indicators suggests these species as a useful focal group in which to study the direct impacts of increasing temperature on endotherms (Oswald & Arnold, 2012). Research on seabirds may help inform best practice for minimising the effects of climate change on terrestrial birds.

Desert and tropical birds are particularly susceptible to increasing temperatures (McKechnie & Wolf 2010; Şekercioğlu *et al.* 2012; Albright *et al.* 2017) and catastrophic mortality events will become much more frequent under future climatic scenarios (McKechnie & Wolf, 2010). For species in tropical regions living closer to their upper thermal limits, even small increases in temperatures may challenge their long-term survival (Khaliq *et al.* 2014). In turn, the combined negative effects of heat waves and droughts on bird abundance are likely to be most pronounced in deserts (Albright *et al.* 2010, 2017). By targeting desert and tropical birds, future research can identify species currently close to their thermal limit as good candidate groups in which to study the direct effects

of temperatures as climates continue to warm (Oswald & Arnold, 2012; Albright *et al.* 2017). By combining this information with indicators of conservation concern (e.g. IUCN Red List status), population change or taxonomic distinctiveness (e.g. Evolutionarily Distinct and Globally Endangered [EDGE] species: https://www.edgeofexistence.org/species/), species in need of rapid, targeted research and management would likely be identified.

Finally, urban environments provide an important opportunity to study heat stress. Cities host vast avian communities supported by access to breeding or roosting habitat and food (Marzluff, 2001). In temperate and sub-polar regions, they also benefit birds via urban heat island effects, allowing for lower thermoregulatory costs during the colder months of the year. In the context of global warming, however, the differential between the cities' core and the non-urban fringe, which can currently reach an average of almost 10 °C during summer daytime (Imhoff *et al.* 2010), may lead to situations of intense heat stress, particularly during heat waves. This is already forecasted to become a major problem for human populations living in the megacities of the future (Matthews *et al.* 2017).

Conclusion

Although avian climate change research has historically been biased towards the study of indirect effects, it is clear that high temperatures and extreme heat episodes are also having profoundly negative direct effects. Our review has revealed a range of physiological and behavioural mechanisms to cope with

extreme heat, yet these are often insufficient to offset deleterious impacts of heat stress and evaporative stress. Our review has highlighted that high temperatures affect individual fitness, either directly via death, or through impacts on eggs and chicks. While large-scale mortality events might be scarce, and therefore have limited population level impacts, they are increasing in frequency with the potential for long-term demographic changes and impacts on species distributions. However, challenges remain in disentangling the direct effects of heat stress from other factors relating to climatic and ecological change. Only by studying the impacts of heat stress more widely, in tandem with other potential factors, can we hope to complete successful multi-species analysis of the direct effects of temperature change. By combining tracking and habitat prediction models, it may be possible to identify the species at greatest risk, enable more accurate modelling of population changes and shifts in geographical distributions, or identify potential thermal adaptations and micro-evolutionary responses to climate change that are already occurring (Oswald & Arnold, 2012). This research can then be incorporated into future species survival models using both the direct and indirect effects of climate change. The results from these will inform predictions about the impact of climate change on species, enabling vital management initiatives needed to combat the effects of anthropogenic climatic change on birds at both a local and global scale.

Table 1. Examples of negative impacts of high temperatures on bird fitness.

	Example	Reference
1. Heat stress		
Mass mortality	In 1932 a heat wave in South Central Australia lead to the deaths of millions of birds due to heat stress.	McKechnie, Hockey & Wolf, 2012
Sub-lethal effects on adults	Ethiopian Bush-crows *Zavattariornis stresemanni* show increased panting behaviour and reduced food intake rate with increasing temperature.	Bladon, 2017
Effects on breeding adults	Heat stress during moult and breeding negatively impacts the survival of adult Little Penguins *Eudyphula minor.*	Ganendran *et al.* 2016
Effects on reproduction mediated by adults	Hoopoe Larks *Alaemon alaudipes* shift their nest sites from open areas to sheltered bushes to reduce the costs of thermoregulation. This in turn increases the risk of nest predation.	Tieleman *et al.* 2008
Effects on eggs and chicks	As temperatures increase above 35°C nest survival decreases for multiple species including Horned larks *Eremophila alpestris* and Western Meadow Larks *Sturnella neglecta.*	Conrey *et al. 2016*
2. Evaporative stress		
Mortality of adults	Stress on cooling and water balance has led to many species dying from hyperthermia through an inability to dissipate water fast enough to regulate body temperature.	McKechnie & Wolf, 2010
Dehydration of breeding adults	Prolonged gular fluttering to regulate body temperature leads to severe dehydration in Cape Gannets *Morus capensis.*	Hochscheid *et al.* 2002
3. Long-term negative impacts		
Population-level effects	By the 2080's, desert bird survival is predicted to be significantly lower, with more frequent nesting failures and mortality events.	McKechnie & Wolf, 2010
Effects on species distribution	Pied Crows *Corvus albus* have shown a South Westwards shift across Southern Africa, with population density declines greatest in areas with a mean annual temperature above 19°C.	Cunningham *et al.* 2016

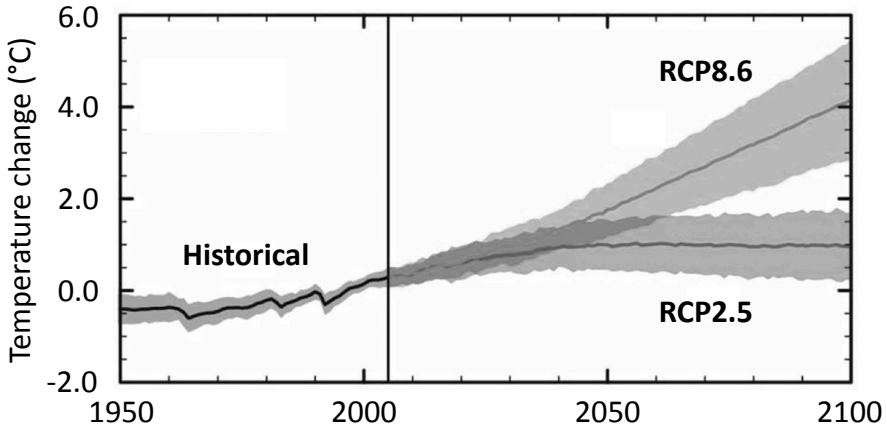

Figure 1. Modelled global average surface temperature change after 2005 (modified from IPCC 2014). Temperature change is expressed as the difference with the average temperature recorded during the 1986-2005 period, here expressed as the baseline (0°C). Two representative concentration pathways (RCP) are shown, describing two climate futures, both of which are considered possible depending on how much greenhouse gases are emitted in years to come. RCP2.5 involves extreme mitigation scenarios aiming to limit the increase in temperature (Van Vuuren *et al.* 2011). RCP8.6 is representative of a high population in absence of climate change policies (Riahi *et al.* 2011). By 2100, RCP2.5 and RCP8.6 predict an increase in global mean temperature of respectively 1°C and 4°C, i.e. an increase of respectively ca. 2°C and 5°C relative to the 1850-1900 period.

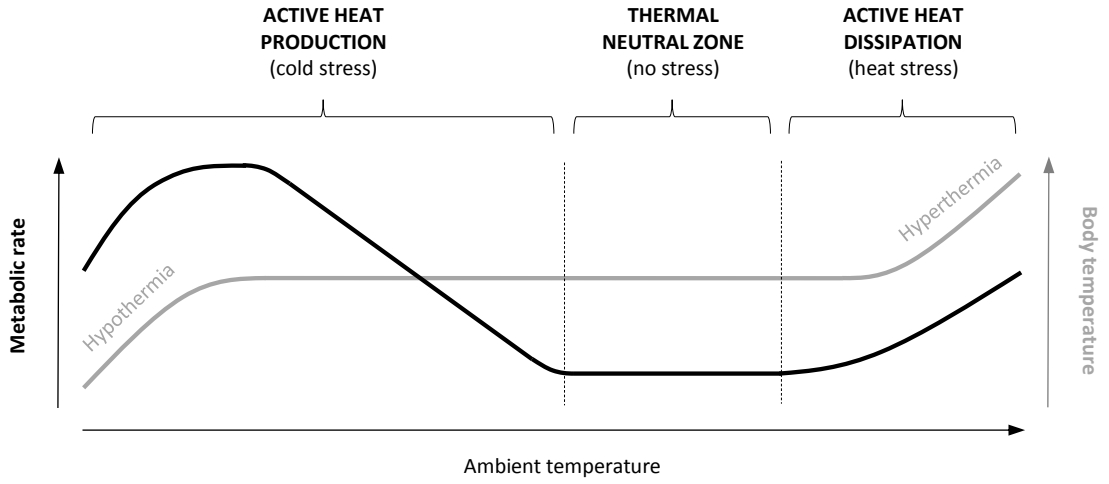

Figure 2. Effect of ambient temperature (arbitrary scale) on metabolic rate and body temperature in endotherms (adapted from Randall *et al.* 2002). In the thermoneutral zone, the body maintains its core temperature solely by regulating dry heat loss, i.e. with changes in vasomotor tone only. Beneath this zone, the body is actively producing heat, mainly metabolically. Above this zone, the body is actively dissipating heat, mainly via evaporative cooling. At the lowest and highest end of the scale, body temperature is either too low, or too high, leading to death.

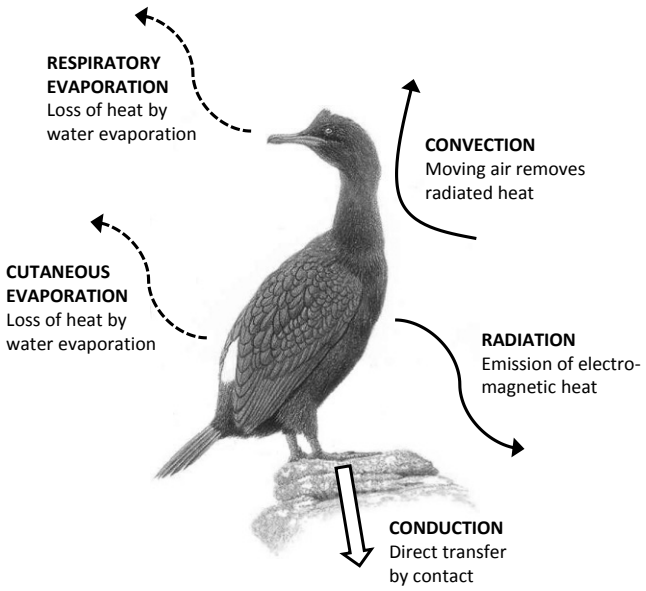

Figure 3. Routes of avian heat loss (adapted from Randall *et al.* 2002). Convection, radiation and conduction transfer heat away from the bird (dry heat loss), but also from the environment to the bird, depending on the thermal gradient with the environment (bird drawing: bank cormorant *Phalacrocorax neglectus* by Robert Cook, Hockey *et al.* 2005).

10°C	20°C	30°C	40°C	50°C	60°C
Birds are not heat stressed	**Birds are mildly heat stressed**	**Birds are heat stressed**	**Birds are heat stressed**	**Birds are severely heat stressed**	☠
	Birds shed excess heat via conduction, radiation, and convection. Some species begin using evaporation. • *postural adjustments*	Conduction, radiation and convection are now less effective. Most species rely also on evaporation. • *postural adjustments* • *birds seek shade* • *adaptive hyperthermia* • *intermittent panting*	Evaporation is now the only route for dumping excess heat. • *birds seek shade* • *adaptive hyperthermia* • *constant panting* • *evaporative stress* • *birds seek water* • *reproductive failure*	Evaporation is not coping with excess heat load in most species. • *direct hyperthermia* • *mass mortality*	

Figure 4. Summary of active routes of heat loss in birds depending on air temperature. The associated level of bird heat stress is indicated, along with relevant behavioural and physiological responses. Temperature thresholds provide a broad scale for understanding bird response, but may vary with environmental conditions, bird size and species. For example, the effects of ambient temperatures >30°C stem primarily from studies on passerine birds from tropical/arid environments.

CHAPTER 2 – THE DIRECT EFFECT OF INCREASING TEMPERATURES ON TWO CLOSELY RELATED SEABIRD SPECIES

Abstract

Increasing temperatures are having major ecological consequences. For endotherms, whilst the indirect effects of higher temperatures are well studied, much less is known about direct effects such as heat stress and water loss. Increasing temperatures may especially impact seabirds, as they forage in cold water yet can be exposed to heat when coming to land to breed. Many seabirds thermoregulate via gular fluttering but this is costly in terms of water loss. Here we use videos to quantify age-specific gular fluttering and estimate water loss for two closely related seabirds: the northern gannet *Morus bassanus,* and the Cape gannet *Morus capensis*. Gular fluttering does not correlate with humidity but increases with temperature for both species, although rates differed by age. Downy chicks have the lowest gular fluttering onset thresholds whilst adult birds have the highest. For chicks, onset thresholds are lower for northern gannets, which may be related to thermal adaptation. Evaporative water loss predicted from gular fluttering was higher for all northern gannet age categories, likely due to their greater mass. Moreover, rates of loss appear unsustainable under climate change scenarios that predict by 2100 downy chicks (the most impacted by evaporative stress) would lose 5.64% (Grassholm) and 4% (Malgas) of daily-ingested water hour^{-1}, leading to critical dehydration in 8 hours. Whilst shifts in breeding phenology in response to increasing temperatures may be possible, if breeding activity is closely linked to peaks in prey availability then overall fitness may be reduced. The results presented here highlight the importance of understanding the direct effects of increasing temperatures on endotherms and the need for further empirical research.

Introduction

Biodiversity is threatened by habitat loss (Brooks *et al.* 2002), invasive species (Gurevitch & Padilla, 2004), overexploitation (Groom, Meffe & Carroll, 2006), pollution (Derraik, 2002) and anthropogenic climate change (Thomas *et al*. 2004). Global temperatures are increasing at historically unprecedented rates (Jones *et al*. 2007, Pachauri *et al*. 2014) with average temperatures predicted to rise 2.2°C by 2100 (IPCC, 2013). Increased frequency of extreme weather events such as heat waves are also forecast (IPCC, 2013).

Globally birds are among the best-studied and monitored organisms, occurring in almost all environments (Pearce-Higgins & Green, 2014). Of the 9856 birds species assessed by the International Union for Conservation of Nature (IUCN) 35% are considered to be vulnerable to the impacts of climate change (Jenouvrier, 2013). Many have shown responses to temperature change, including changes in distribution (Milne *et al.* 2015), phenology (Lehikoinen & Sparks, 2010), demographic processes (Pearce-Higgins & Green, 2014), and behaviour (Walther *et al.* 2002; Parmesan, 2006). Responses can broadly be categorised into two distinct types, direct and indirect effects. The direct effects are the direct processes where by temperature changes affect the environment, behaviour, growth and development rates of birds (Ockenden *et al.* 2014), for example altering species' water balance (Albright *et al*. 2017) and patterns of thermoregulation (du Plessis *et al.* 2012). The indirect effects are the mechanisms by which the effects of increasing temperatures on one species are interceded via effects on another species (Ockenden *et al.* 2014). To date, a large body of work has focused on indirect effects, including the altered

availability, abundance and quality of food (Cohen *et al.* 2014), interspecific and intraspecific interactions (Parmesan, 2006, Tylianakis *et al.* 2008) and the availability, suitability and quality of habitat (Galbraith *et al.* 2002). Direct effects however, have received much less attention (but see: Oswald & Arnold, 2012). This may be because it is often assumed that indirect effects are of greater importance (Ockenden *et al.* 2014) as birds can maintain stable body temperatures independently from ambient conditions (McNab, 2012).

The Thermoneutral zone (TNZ) is the range of temperatures over which energy used for thermoregulation and evaporative water loss is at a basal level and varies among species (Romanovsky, Ivanov & Shimansky, 2002; Blache, Terlouw & Maloney, 2011; Oswald & Arnold, 2012). When temperatures exceed the TNZ, birds endeavor to lose heat via physiological and behavioural mechanisms. Consistent temperatures outside a species' TNZ can compromise normal body function and result in heat stress (McNab, 2002). Energy and time previously invested in growth or reproduction must be re-allocated to active thermoregulation, known as the 'biological cost of stress' (Moberg, 2000; McNab, 2002). Prolonged elevation of body temperature outside of the TNZ can lead to hyperthermia and an increased risk of death (Angilletta, 2009; Speakman & Król, 2010; Oswald & Arnold, 2012).

Seabirds

Seabirds are amongst the most vulnerable of all birds (Barbraud *et al.* 2012; Sydeman *et al.* 2012) and knowledge of their ecology, behaviour and conservation status is comprehensive (Schreiber & Burger, 2001; Croxall, Trathan, & Murphy, 2002). Furthermore, they are key bioindicators of the health

of marine ecosystems (Piatt, Sydeman & Wiese, 2007; Durant *et al.* 2009; Grémillet & Charmantier, 2010). Predominantly impacted by climate change through changes in prey availability and pelagic habitat (Sydeman, Thompson, & Kitaysky, 2012; Franklin *et al.* 2014), seabirds must also balance the conflicting thermoregulatory constraints of foraging at sea - requiring highly insulative plumage, and nesting on land – where exposure to high levels of solar radiation and heat loads can occur (Oswald & Arnold, 2012). This makes them extremely vulnerable to overheating and heat stress (Kearney, 2011). Further constraint occurs during incubation and chick-rearing when adults alternate nest attendance and have limited access to food, water or protection from the elements (Oswald *et al.* 2011).

Physiological and behavioural adaptations enable seabirds to minimise heat gain and maintain thermal homeostasis. Feathers facilitate heat loss via ptiloerection (compression and elevation of plumage, Bartholomew & Dawson, 1979). Additionally, plumage colour and structure can determine heat load from solar radiation, with loads typically greater for birds with darker plumage (Wolf & Walsberg, 2000, Hochscheid *et al.* 2002). Moving to a less exposed location can minimise heat load, yet is not always possible due to costly trade-offs. For example, bathing and soaring require leaving nesting sites for extended periods, exposing chicks to solar radiation and increased predation risk (Oswald & Arnold, 2012). Other thermoregulatory behaviours include: postural adjustments (Ward *et al.* 2008), shading (Brown & Downs, 2003), increasing blood flow to the legs and feet (Bernstein, 1974), exposing legs, feet and thinly feathered areas to air flow (Howell & Bartholomew, 1961), excreting on their own legs (Kahl, 1963), extending the wings away from the body (Cook &

Leblanc, 2007) and increased ventilation via panting or gular fluttering (vibration of the hyoid apparatus) (Dawson, 1982). Gular fluttering results in high levels of evaporative water loss (Lasiewski & Snyder, 1969; Hochscheid *et al.* 2002) and is typically used when other mechanisms for heat dissipation are not possible, for instance during incubation or chick-rearing (Dawson, 1982). Subsequently, gular fluttering is a good indicator of heat stress.

Aims of the study

This study aims to quantify the degree of heat stress experience by two seabirds, the northern gannet *Morus bassanus* found in generally cool temperate climes (breeding season temperature; Low - 10°C, High - 27°C, Average - 16°C, (Thorsen, 2018a)) and the Cape gannet *Morus capensis* that breeds in a warmer Mediterranean climate in South Africa (breeding season temperature; Low - 11°C, High - 31°C, Average - 21°C (Thorsen, 2018b)). Specifically, I aim to establish the proportion of time spent gular fluttering in relation to ambient temperature and how this varies across species and age. Moreover, using gular fluttering rates in tandem with models of evaporative water loss I want to establish the variation in the cost of heat stress through estimations of evaporative water loss. Finally I aim to ascertain how patterns of gular fluttering and water loss might alter in the face of a warming climate.

Materials and Methods

Study Species

The northern gannet is the largest member of the Suildae, distributed throughout the North Atlantic, with breeding colonies across the U.K., Canada, Europe and Scandinavia (Nelson, 2002). Adults are 81-110cm in length, weigh 2.2-3.6kg and have a wingspan of 165-180cm whilst immature birds can weigh up to 4kg before fledging (Nelson, 2002). Northern gannets have an estimated population size of 1,500,000-1,800,000 individuals and are listed as least concern by the International Union for the Conservation of Nature (IUCN) (BirdLife International, 2016). Chicks are initially featherless and completely black before developing a layer of thick white downy feathers (Nelson, 2002). As they age, they develop dark brown/black feathers, with those that are near to fledging almost entirely dark brown/black. Adult birds are white with dark brown/black wingtips and incubate a single egg for 42-46 days. Chicks fledge after 11-12 weeks (Nelson, 2002).

Cape gannets are restricted to Southern Africa, breeding on six islands off the coast of Namibia and South Africa (BirdLife International, 2017). Adults measure 84–94 cm in length, weigh 2.6kg on average and have a wingspan of 171–185 cm (BirdLife International, 2017). Cape gannets have an estimated population of 300,000 individuals and are classified as vulnerable by IUCN (BirdLife International, 2017), by virtue of their restricted breeding range, overfishing of prey and pollution (Crawford *et al.* 2007). Adults are white with dark brown/black tail feathers, primaries and secondaries. Chicks are featherless and black before developing white downy feathers. They then

develop dark brown/black feathers and are almost entirely dark brown/black upon fledging (Crawford, 2005). Eggs are incubated for 42 to 46 days, with fledging at 95 to 105 days post hatching (Crawford, 2005).

Study Sites

For northern gannets data collection took place on RSPB Grassholm, Wales situated off the southwestern coast of Pembrokeshire (51.7307°N, 5.4800°W) (See appendices for dates). It is the third largest gannet colony in the U.K., home to over 36,000 breeding pairs. Data collection for Cape gannets took place on Malgas Island, South Africa , located in the entrance to Saldanha Bay, in the Western Cape province of South Africa (33.0528°S, 17.9254°E) (See appendices for dates). Malgas is part of the West Coast National Park and home to over 20,000 breeding pairs of Cape gannets (approximately 12% of the global population).

Recording Climate Data

Ambient temperature, operative temperatures and relative humidity (%RH) were recorded at each colony. Operative temperature is a measure of the temperature experienced by the birds in the absence of thermoregulatory behaviours and metabolic processes. It can therefore be distinguished from ambient temperature, which is the temperature of the environment. Temperatures were recorded using thermochron iButtons (model no. DS1920) whilst humidity data was recorded using hygrochron iButtons (model no. DS1923). iButtons were factory calibrated to a resolution of 0.0625°C and 0.04%RH and were programmed using OneWireViewer from Maxim Integrated Products Inc to take readings every 10 minutes. To measure ambient

temperature and humidity, two thermochron and one hygrochron iButton were suspended inside a cylindrical Stevenson's screen (15cm high, 13cm diameter). Two screens were deployed at each colony using a 50cm piece of threaded rod. Historically, operative temperature models were constructed using a taxidermic mount placed over a hollow copper cast of the animal (Bakken, 1992). In a study by Walsberg and Weathers (1986) a comparison of operative temperature estimates between mounted specimens and thermometers inside painted metal spheres found an average difference of less than 2°C in long term studies. Consequently, our study used painted metal spheres due to their durability and ease of transport. Models were assembled from two copper alloy half-spheres (0.9 mm thickness), attached together with TESA adhesive tape. Each sphere was pierced with three holes to enable two thermochron iButtons to be suspended inside, avoiding contact with the sphere. A 50cm threaded rod was inserted into the third hole, allowing the sphere to be mounted in the ground at a height of approximately 30cm. All holes were sealed with TESA tape to prevent airflow. In total, four models were constructed, two were painted matte black and two matte white, mimicking the absorbance and reflectance properties of gannet plumage. The size of the spheres (12.5cm circumference) was determined using adult Cape gannet chest circumferences prior to fieldwork. Due to limited resources, the spheres for Cape gannets were also used for northern gannets. Spheres were positioned as close as possible to each colony without disturbing the birds (See appendices).

Recording Gannet Behaviour

To record behaviour, four Sony handycams (2 CX190HD cameras and 2 SX22E cameras) were set up across the colony. Filming took place for 10

minutes every hour (see appendices for filming dates and durations). Each 10-minute clip was synchronised to the iButton readings for that time period. Upon review, only footage captured by the two High Definition cameras was of sufficient quality for behavioural analysis. Using imageJ (Schneider, Rasband & Eliceiri, 2012) birds across four age classes; chick (all downy feathers), chick (>50% black feathers), chick (all black feathers) and adults, were selected for analysis. The event recorder JWatcher 1.0 was used to record behaviours with each bird analysed individually as a separate clip (Blumenstein *et al.* 2006). The Focal Master tab was used to create a list of behaviours with a key code assigned to each and were defined as mutually exclusive or capable of co-occurring using the Focal Analysis Master tab. Video footage was run on QuickTime Media Player whilst the Data Capture tab from JWatcher ran simultaneously. When a behaviour occurred, the corresponding key press was recorded, along with the associated behaviour and the time. Behaviour durations and time budgets were then calculated using JWatcher's Analysis tab and saved as a results.csv file. A team of trained undergraduate students assisted with video analysis. For the purpose of this study the sole behaviour incorporated into analysis was gular fluttering.

Data Analysis

Temperature data

Thermochron iButton recordings from each sphere were averaged to obtain one value for operative white and black temperature for each 10-minute video segment. The same was done for the thermochron and hygrochron iButtons in the Stevenson screens to give values for ambient temperature and humidity

respectively. Simple linear regressions were then fitted to explain operative temperature as a function of ambient temperature for adults and black chicks of each species using the lm function in R (R Core Team, 2017).

Gular fluttering correlations

Gular fluttering was recorded as a continuous variable, calculated as the proportion of time that a bird spent gular fluttering in each 10-minute sampling interval, with “0” being no gular fluttering through to “1” constant gular fluttering. Simple linear regressions were carried out to investigate gular fluttering as a function of ambient temperature and humidity. For these, all data for species and age categories were combined and fitted using the lm function in R (R Core Team, 2017).

Gular fluttering models

Whilst gular fluttering proportion was a continuous variable, the distribution of gular fluttering data were highly skewed so here a binary transformation was used, with proportion ≥ 0.5 given a value of “1” and < 0.5 = “0”. This enabled each observation to be classified as gular fluttering or not. To determine which factors influenced gular fluttering, a generalised linear model was fit using the lme4 package in R (Bates *et al.* 2014), with gular fluttering as the binary response variable and ambient temperature, age, site (a proxy for species) and humidity as fixed effects. The selected covariates and the interactions between them were fitted as fixed effects. The best models were selected based upon AICc values, using the dredge function from the MuMIn package in R (Barton, 2016). A ΔAICc = 2 value was used to define the set of best-supported models and the fit of the model was determined using the percentage of deviance

explained, given as (deviance of the null model – deviance of the explained model) / deviance of the null model x 100.

Gular fluttering threshold temperatures

To determine temperature thresholds for gular fluttering onset, heat stress (100% gular fluttering) and the rate of increase in gular fluttering as a function of temperature, the moult package in R was used (Erni *et al.* 2013). This package estimates the start and duration of moult in birds, where there is no feather growth until a threshold is reached, at which point feathers grow until the moult is complete, similar to gular fluttering distribution. In this analysis, the proportion of time spent gular fluttering was continuous and not a binary variable, it could vary anywhere between "0" for no gular fluttering to "1" for gular fluttering during the whole 10-minute segment. Here gular fluttering proportion was the response variable, ambient temperature the main explanatory variable, and age and site (representing species) as fixed effects influencing the threshold (temperature at which gular fluttering begins) and the slope (the rate at which gular fluttering increases with temperature).

Modelling evaporative water loss

To estimate water loss via gular fluttering, a model modified by Hochscheid *et al.* (2002) was used. This model estimates water lost $hour^{-1}$ of gular fluttering as a percentage of the total amount of daily-ingested water, assumed to be derived entirely from food. To determine daily food intake (DFI) for each age category and species, the following equation was used:

DFI = Field metabolic rate (FMR) / energetic value of prey x assimilation rate of prey

To calculate field metabolic rate in kJ/day (FMR) the following allometric equation was used (Ellis & Gabrielsen, 2002):

$$FMR\ (kJ/d) = 3.9 \times mass\ (g)^{0.871}$$

where 3.9 and 0.871 are constants. The average mass values used for each age category were as follows: For northern gannets; adults 3kg, black chicks 3.7kg, chicks >50% black 3.2kg and downy chicks 1.6kg (Montevecchi *et al.* 1984, Nelson, 2002). For Cape gannets: adults 2.6kg, black chicks 3kg, chicks >50% black 2.5kg and downy chicks 1.2kg (Mullers *et al.* 2009, Navarro *et al.* 2015). The average energetic value of prey was taken to be 7kJ/g for northern gannets (Barrett, 2016) and 6.5kJ/g for cape gannets (Balmelli & Wickens, 1994) with an assimilation rate of 77.25% (Visser, 2002). Ingested water was estimated to be approximately 80% of the DFI (Hochscheid *et al.* 2002). Values for evaporative water loss (EWL) were taken from Lasiewski *et al.* (1966) and are given in mg H_2O /g of body weight/hour. Values are 1.82 mg H_2O/g for basal EWL with no gular fluttering, 9.85 mg H_2O /g when gular fluttering 50% of the time, and 12.56 mg H_2O /g when gular fluttering 100% of the time. Using estimated daily water intake and calculated EWL rates, the total percentage of ingested water lost $hour^{-1}$ when not gular fluttering, gular fluttering 50% and 100% of the time were calculated for all age categories, across both species. The values for mass, prey energetics and assimilation rates were chosen to replicate the values for each species and age category in the study as closely

as possible. Consequently, whilst there is likely to be some degree of error, the values generated from the equations provide a best guess estimation of the rates of evaporative water loss across age and species in this study (see appendices for FMR, DFI and EWL values for each age category and species).

Modelling impacts of climate change on gular fluttering and water loss

An average global temperature increase of 0.5°C by 2035, 1.3°C by 2065, and 2.2°C by 2100 is currently projected by climate change models (IPCC, 2013). To calculate future water loss in nesting northern and cape gannets, gular fluttering proportion was first modelled as a function of the current average ambient temperature, using average daily mean and maximum temperatures during the fieldwork period. Proportions were then modelled applying projected temperature increases. These values were then converted to the corresponding amount of ingested water lost $hour^{-1}$, giving EWL under the future climate change scenario.

Results

In total, video segments for 335 birds from Grassholm (131 chicks (downy), 75 chicks (>50% black), 18 chicks (black) and 111 adults) and 1407 birds from Malgas (321 chicks (downy), 309 chicks (>50% black), 308 chicks (black) and 469 adults) were analysed. Upon watching the footage back it was clear that across both species, birds were rarely seen to have both their head up and beak open without gular fluttering.

Temperature

On Grassholm mean ambient temperature was 19.98°C, mean operative temperature for the black orb was 27.00°C and 20.47°C for the white orb. At Malgas mean ambient temperature was 20.41°C, mean operative temperature for the black orb was 29.16°C and 20.89°C for the white orb. Linear regressions found a strong positive relationship for both black and white orbs at both colonies (Figure 5). However, here subsequent analyses will use values for ambient temperature because operative temperature experienced by chicks with a mixture of white and black feathers would not be accurately represented.

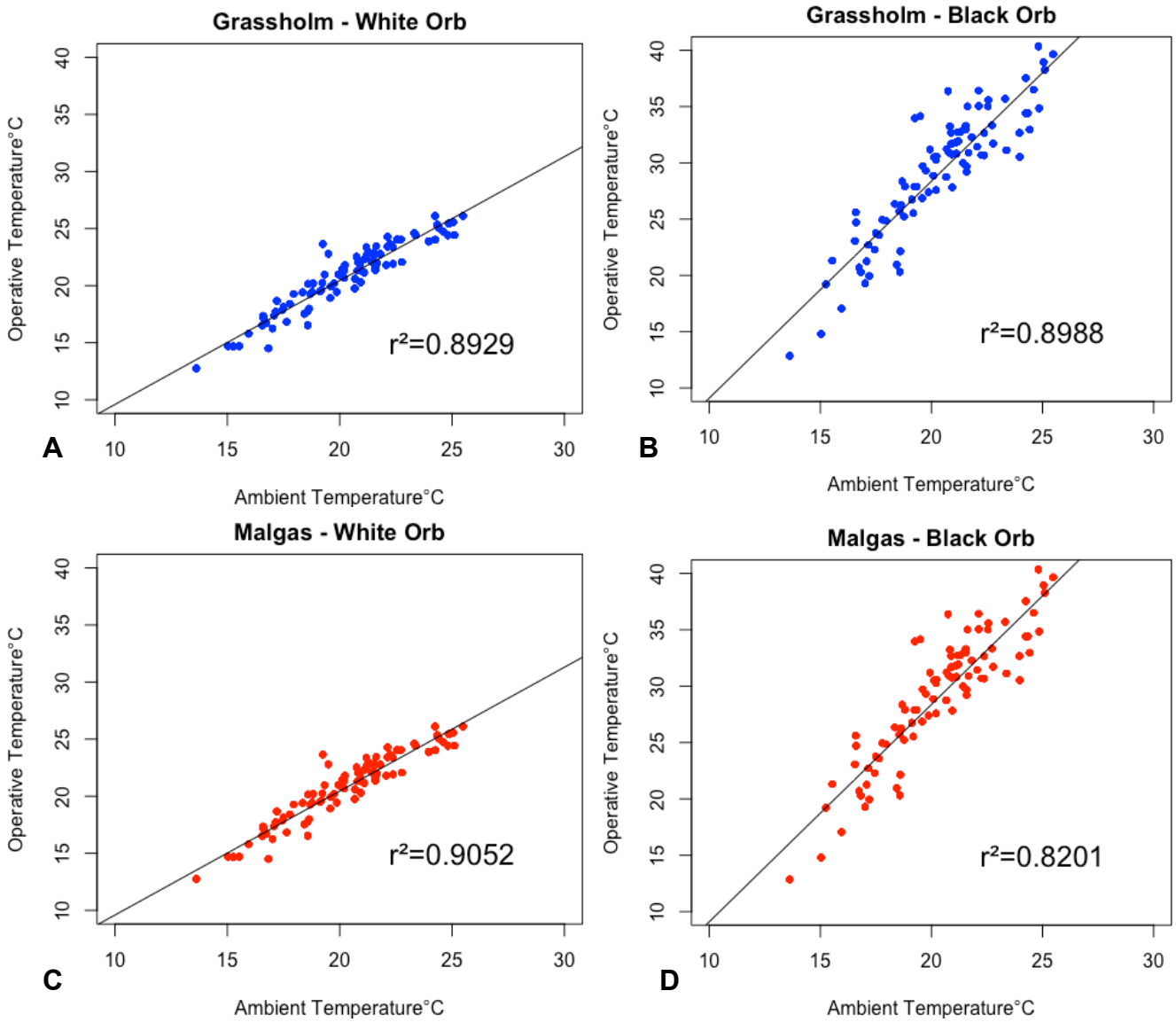

Figure 5. Linear regression showing the relationship between ambient (T_a) and operative (T_o) temperature for white (**A** and **C**) and black (**B** and **D**) painted copper orbs, across both breeding colonies (Grassholm: **A** and **B**. Malgas: **C** and **D**). At Grassholm, the relationship between T_o (white orb) and T_a was: $T_o = 1.041*T_a - 0.325$, $F_{1,333} = 2787$, $p < 0.0001$, $r^2 = 0.8929$, $N = 335$ (Figure 4**A**), and between T_o (black orb) and T_a was: $T_o = 2.080*T_a - 14.559$, $F_{1,333} = 2966$, $p < 0.0001$, $r^2 = 0.8988$, $N = 335$ (Figure 4**B**). At Malgas, the relationship between T_o (white orb) and T_a was: $T_o = 1.086*T_a – 1.267$, $F_{1,1405} = 1.343e+04$, $p < 0.0001$, $r^2 = 0.9052$, $N = 1407$ (Figure 4**C**), and between T_o (black orb) and T_a was: $T_o = 1.927*T_a – 10.167$, $F_{1,1405} = 6410$, $p < 0.0001$, $r^2 = 0.8201$, $N = 1407$ (Figure 4**D**).

Gular fluttering correlations

Simple linear regressions revealed that for combined data across both sites and all age categories, there was a significant positive relationship between gular fluttering proportion and ambient temperature (Figure 6A) but a non-significant negative relationship between gular fluttering proportion and humidity (Figure 6B)

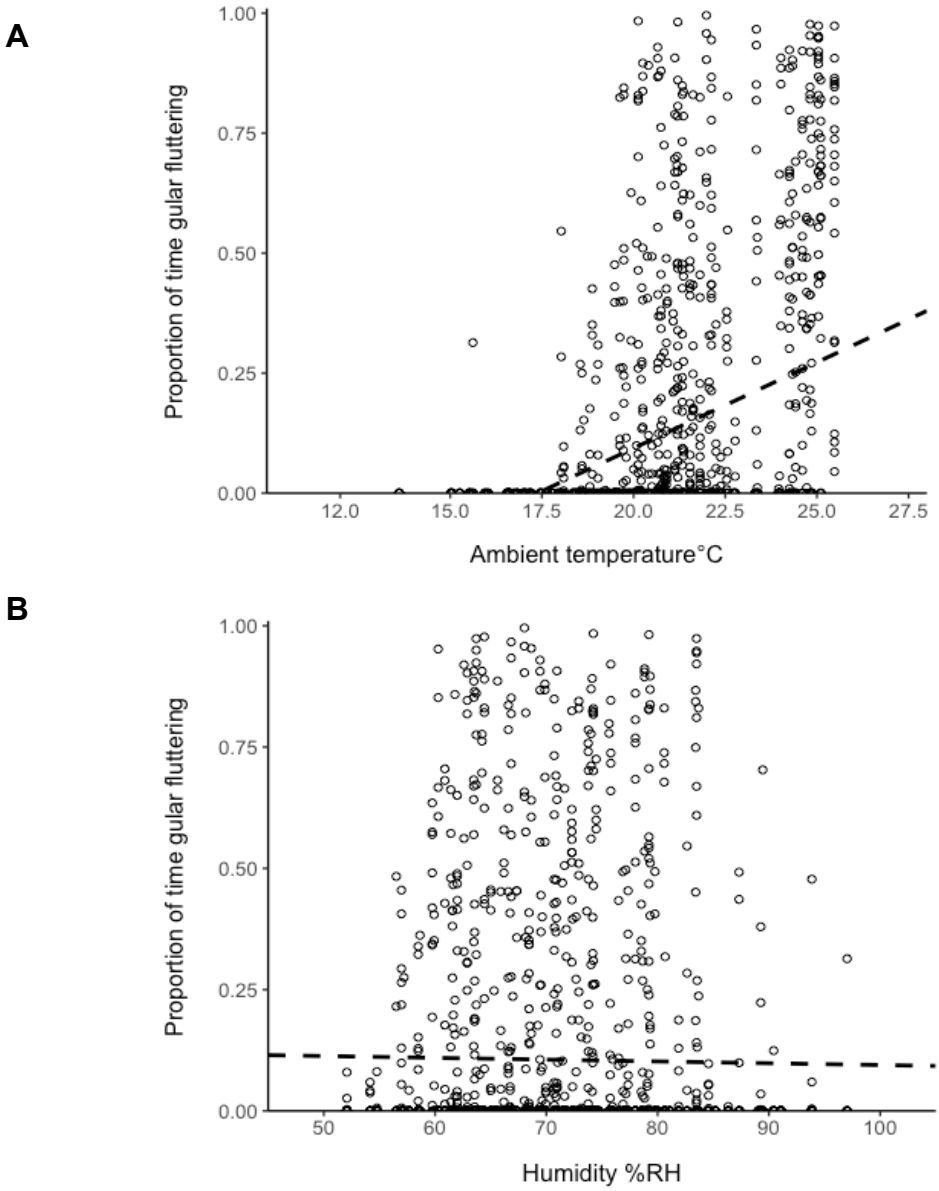

Figure 6 (A) The relationship between the proportions of time spent gular fluttering (Prop. GF) and ambient temperature (T_a) (°C) for gannets across both colonies: Prop. GF = 0.036*T_a - 0.622, $F_{1,333}$ = 344.1, p <0.0001, r^2 = 0.165. **(B)** The relationship between gular fluttering proportion and humidity (%RH) for gannets across both colonies: Prop. GF = -0.0004*Humidity + 0.13, $F_{1,1740}$ = 0.396, p = 0.527, r^2 = -0.0003

Gular fluttering models

The best-fit model of binary gular fluttering included age, site, ambient temperature and humidity as main effects, as well as the interactions between age and site, age and humidity, site and humidity and finally ambient temperature and humidity (Table 2). After multi-model analysis, the best candidate model explained 34.23% of the deviance. In all models, age, site, ambient temperature and humidity were selected as important variables in explaining gular fluttering proportion. The interactions between age and site, age and humidity and ambient temperature and humidity were also selected as important. Here, further analysis focused on ambient temperature, age and site.

Table 2. Generalized linear model comparisons using Aikake's Information Criterion corrected for small sample sizes (AICc) relating binary gular fluttering response (GF) to age, site, ambient temperature (Ta) and humidity (Hu). For each model, values are given for degrees of freedom (df), AICc, ΔAIC, AIC weight (wAIC) and percentage of deviance explained (%DE).

Candidate Model	df	AICc	ΔAIC	wAIC	% DE
GF~ age+site+Ta+Hu+age*site+age*Hu+site*Hu+Ta*Hu	15	733.5	0.00	0.309	34.22
GF~ age+site+Ta+Hu+age*site+age*Hu+site*Ta+Ta*Hu	15	735.0	0.48	0.243	34.17
GF~ age+site+Ta+Hu+age*site+age*Hu+Ta*Hu	14	734.3	0.76	0.211	33.95
GF~ age+site+Ta+Hu+age*site+age*Hu+site*Ta+site*Hu+Ta*Hu	16	735.54	1.85	0.122	34.23
GF~ age+site+Ta+Hu+age*site+site*Hu+Ta*Hu	12	735.5	1.98	0.115	33.46
GF~NULL	1	1070.9	337.35	0.000	-

Gular fluttering thresholds and evaporative water loss

Models were created to predict threshold temperatures for gular fluttering onset, heat stress (Figure 7) and rates of evaporative water loss based upon gular fluttering 0%, 50% (intermittent) and 100% (stressed) (Figure 8). Models were

used to compare variation across each age category and site. An age category is deemed stressed when half the birds are gular fluttering all of the time (represented by 1.0, where birds are as likely to be gular fluttering as not gular fluttering).

Chicks (downy)

At both colonies, gular fluttering occurs at the lowest temperatures for chicks (downy): Grassholm - 18.345±0.639°C, Malgas - 22.096±0.780°C (Figure 7A, Table 3). Beneath these, basal EWL is 0.82% hour-^{1} at Grassholm and 0.73% hour-^{1} at Malgas. At 21.86°C, gular fluttering proportion increase to 50% at Grassholm, resulting in EWL of 4.42% hour^{-1} and 22.1% after 5 hours (Figure 8A). In contrast, at Malgas 50% gular fluttering occurs at 25.55°C, with EWL of 4% hour^{-1} and 20% after 5 hours (Figure 8A). At Grassholm, 100% gular fluttering and heat stress occur at 25.38°C with EWL of 5.64% hour-1 and 28.2% after 5 hours (Figure 8A). At Malgas 100% gular fluttering and heat stress occur at 29.01°C. Here, EWL is 5.05% hour^{-1}, with chicks (downy) losing 25.25% of their daily-ingested water after 5 hours (Figure 8A).

Chicks (>50% black)

Basal EWL for chicks (>50% black) is 0.89% hour-1 at Grassholm and 0.80% at Malgas. Gular fluttering onset occurs at 19.533±1.017°C at Grassholm and 22.618±1.120°C at Malgas (Figure 7B, Table 3). At 24.52°C, birds at Grassholm will spend 50% of their time gular fluttering, with EWL of 4.84% hour^{-1} and 24.2% after 5 hours (Figure 8B). At Malgas 50% gular fluttering occurs at 25.79°C, with EWL of 4.35% hour^{-1} and 21.75% after 5 hours. At Grassholm, 100% gular fluttering occurs at 29.50°C with EWL at 6.17% hour-^{1} and 30.85%

after 5 hours (Figure 8B). At Malgas 100% gular fluttering occurs at 28.96°C, with EWL of 5.56% hour^{-1} and 27.8% after 5 hours (Figure 8B).

Chicks (black)

EWL is highest for chicks (black) (Table 3). At Grassholm, basal EWL is 0.91% hour^{-1} prior to gular fluttering onset at 22.499±1.905°C. At Malgas basal EWL is 0.82% hour^{-1} with gular fluttering onset at 23.748±2.015°C (Figure 7C, Table 3). At Grassholm 50% gular fluttering occurs at 25.53°C, with EWL at 4.93% hour^{-1} and 24.65% after 5 hours (Figure 8C). 50% gular fluttering occurs at 26.28°C at Malgas, with EWL at 4.45% hour^{-1} and 22.25% after 5 hours (Figure 8C). Heat stress occurs at Grassholm at 28.57°C, with EWL of 6.28% hour^{-1} and at 28.81°C, with EWL of 5.68% at Malgas. After 5 hours of heat stress chicks (black) will lose 31.4% of their water at Grassholm and 28.4% at Malgas (Figure 8C).

Adults

Gular fluttering thresholds are highest for adults. At Grassholm basal EWL rate is 0.89% hour^{-1}, with gular fluttering occurring above 25.377±1.484°C. At Malgas basal EWL is lower, 0.81% hour^{-1}, occurring at 24.943±1.570°C (Figure 7D, Table 3). At Grassholm 50% gular fluttering occurs at 27.96°C, EWL increases to 4.80% hour^{-1} and 24% after 5 hours (Figure 8D). At Malgas, 50% gular fluttering occurs at 27.13°C and EWL is 4.37% and 21.85% after 5 hours (Figure 8D). Heat stress occur at Grassholm at 30.55°C, with 6.11% EWL hour-1 and 30.55% loss after 5 hours. For adults at Malgas, heat stress occurs at 29.33°C. Here EWL is 5.57% hour^{-1} and 27.85% after 5 hours of continuous gular fluttering (Figure 8D).

Onset thresholds for chicks (downy), chicks (>50% black) and chicks (black) are lower at Grassholm than Malgas (Figure 7A-C, Table 3). Chicks (downy) and chicks (black) become thermally stressed at lower temperatures at Grassholm than at Malgas whilst chicks (>50% black) and adults at Grassholm do so at higher temperatures (Figure 7, Table 3). Gular fluttering and EWL increased with temperature, with rates of EWL higher at Grassholm and EWL highest for chicks (black) at both sites (Figure 7).

Table 3. Ambient temperature thresholds (±95% Confidence Intervals) for gular fluttering onset and heat stress for each age category across both sites.

AGE	**SITE**			
	Grassholm	**Malgas**	**Grassholm**	**Malgas**
	GF Start T_a (°C)	**GF Start T_a (°C)**	**GF Stressed T_a (°C)**	**GF Stressed T_a (°C)**
Chicks (downy)	18.35 ± 0.64	22.10 ± 0.78	25.38 ± 0.64	29.01 ± 0.78
Chicks (>50% black)	19.53 ± 1.02	22.62 ± 1.12	29.499 ± 1.02	28.96 ± 1.12
Chicks (black)	22.50 ± 1.91	23.75 ± 2.02	28.57 ± 1.91	28.81 ± 2.02
Adult	25.38 ± 1.49	24.94 ± 1.57	30.55 ± 1.48	29.44 ± 1.57

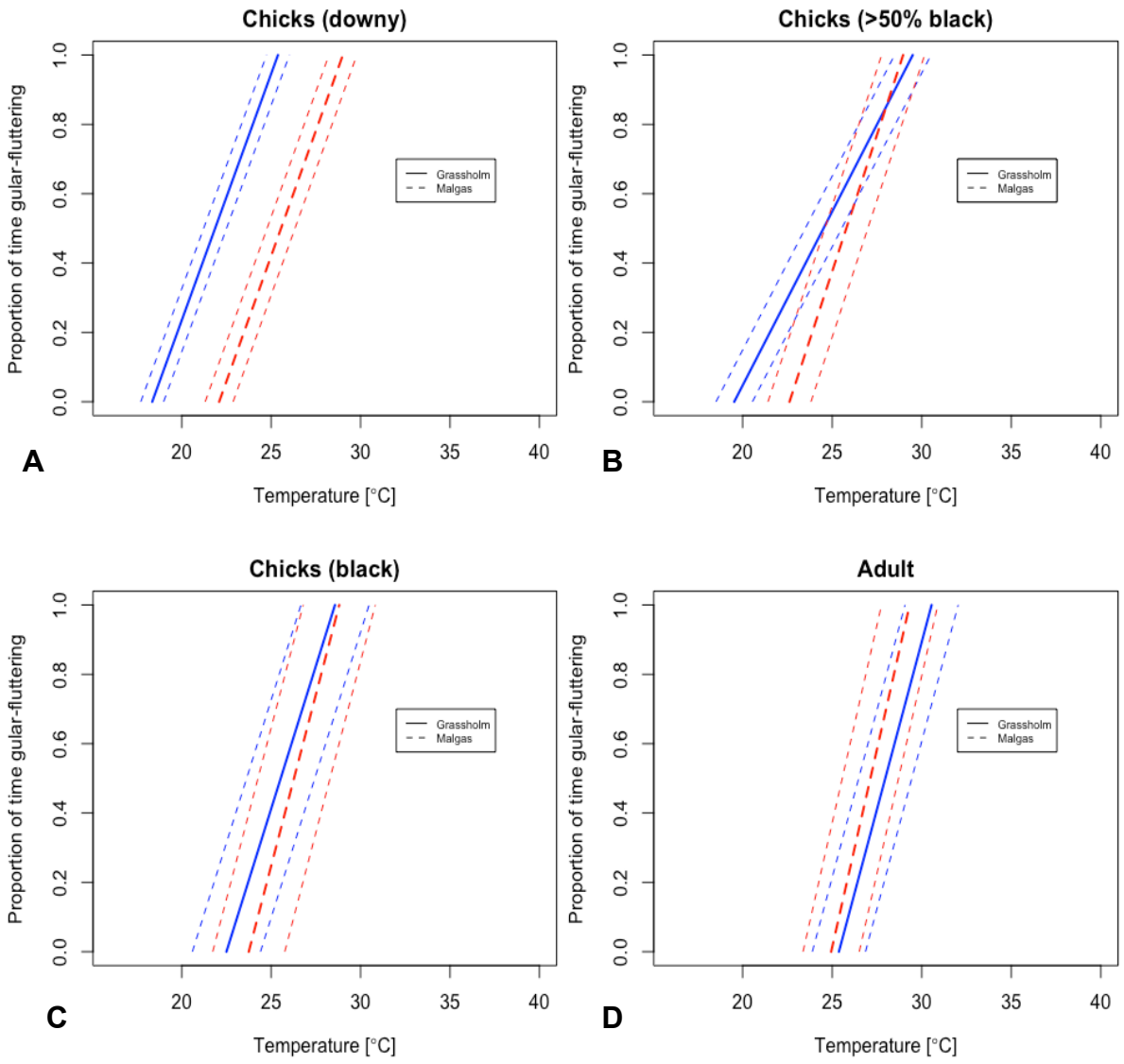

Figure 7. Gular fluttering proportion for Chicks (downy) (A), Chicks (>50% black) (B), Chicks (black) (C) and Adult gannets (D) at Grassholm (Blue solid line) and Malgas (Red dashed line) as a function of ambient temperature (±95% Confidence Intervals).

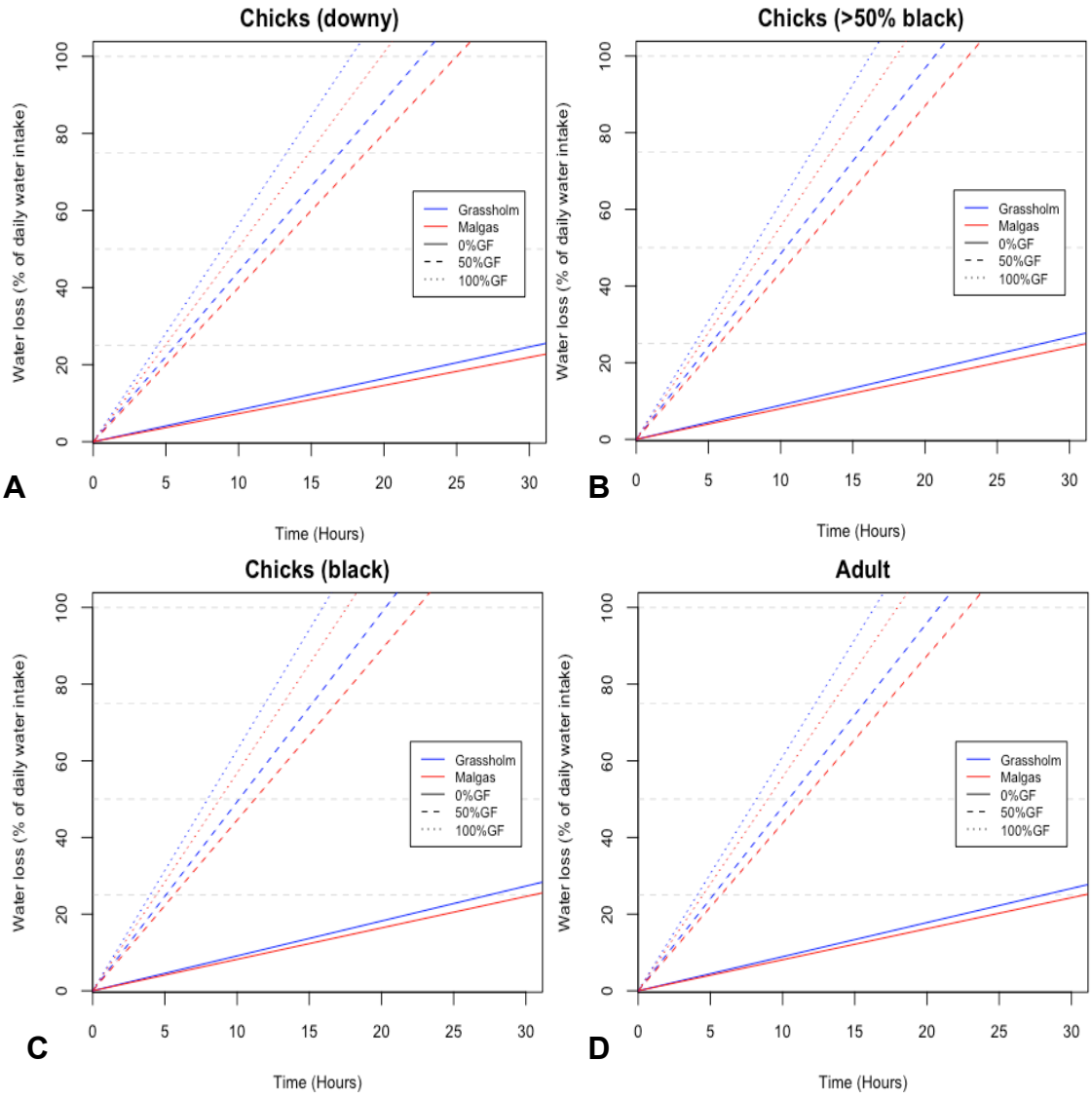

Figure 8. Theoretical water loss as a percentage of daily-ingested water and time spent gular fluttering for Chicks (downy) (A), Chicks (>50% black) (B), Chicks (black) (C) and Adult gannets (D) at Grassholm (Blue) and Malgas (Red). Solid line: 0% Gular fluttering, Dashed line: 50% Gular fluttering and Dotted line: 100% Gular fluttering.

Modelling impacts of climate change on gular fluttering and water loss

Using current climate change models, based upon temperature increases projected by the IPCC (2013) of +0.5°C by 2035, +1.3°C by 2065 and +2.2°C by 2100, predictions for gular fluttering proportions and EWL can be made using mean and maximum ambient temperatures recorded at each colony.

Using mean temperature projections, by 2100 gular fluttering proportion for (downy) and chicks (>50% black) at Grassholm will increase to 0.55 and 0.265 respectively. A 2.2°C temperature increase will not result in gular fluttering thresholds being met for chicks (black) and adults. At Malgas mean temperature projections will result in an increase to 0.08 for chicks (downy) but onset will not occur for all other age categories (Table 4).

Maximum temperature projections show an increase to 1 (constant) gular fluttering for chicks (downy) at Grassholm and to 0.81 at Malgas by 2100. For chicks (>50% black) gular fluttering proportion will increase to 0.74 at Grassholm and 0.80 at Malgas and for chicks (black) to 0.73 at Grassholm and 0.78 at Malgas. For adults, gular fluttering proportion will increase to 0.3 at Grassholm and 0.63 at Malgas (Table 4).

Table 4. Current and projected gular fluttering proportions according to temperature increases projected by the IPCC, based upon mean and maximum temperatures during the fieldwork seasons at Grassholm (G) and Malgas (M).

By 2100 EWL for chicks (downy) at Grassholm, will increase to 4.53% hour^{-1}

Age	Site	T_a	Current	Projected 2035	Projected 2065	Projected 2100
Chicks (downy)	G	Mean	0.23	0.30	0.42	0.55
		Max	0.91	0.98	1	1
	M	Mean	0	0	0	0.08
		Max	0.49	0.56	0.68	0.81
Chicks (>50% black)	G	Mean	0.05	0.10	0.18	0.27
		Max	0.52	0.57	0.65	0.74
	M	Mean	0	0	0	0
		Max	0.45	0.53	0.66	0.80
Chicks (black)	G	Mean	0	0	0	0
		Max	0.37	0.45	0.58	0.73
	M	Mean	0	0	0	0
		Max	0.34	0.44	0.60	0.78
Adult	G	Mean	0	0	0	0
		Max	0	0	0.12	0.30
	M	Mean	0	0	0	0
		Max	0.12	0.24	0.42	0.63

under mean projections and 5.64% under maximum temperature projections. At Malgas, EWL will increase to 1.22% under mean projections and to 4.65% under maximum projections (Table 5A). For chicks (>50% black) at Grassholm, EWL will increase to 2.99% by 2100 under mean projections and 5.48% under maximum projections. For chicks (>50% black) at Malgas, EWL will remain at the current rate of 0.80% by 2100 under mean projections and increase to 5.07% hour^{-1} under maximum temperature projections (Table 5B).

Table 5. EWL hour^{-1} as a percentage of daily-ingested water for chicks (downy) **(A)** and chicks (>50% black) **(B)** across both sites, at ambient temperatures for gular fluttering threshold (0% GF), intermittent gular fluttering (50% GF) and

heat stress (100% GF). EWL values are also shown for current mean and maximum ambient temperatures and under climate change projections.

A

Grassholm Chicks (downy)							Malgas Chicks (downy)					
	Mean			Max				Mean			Max	
	Amb	EWL		Amb	EWL			Amb	EWL		Amb	EWL
0% GF	18.35	0.82	0% GF	18.35	0.82		Current	20.41	0.73	0% GF	22.09	0.73
Current	19.98	2.49	50% GF	21.86	4.42		2035	20.91	0.73	Current	25.48	3.93
2035	20.48	3.00	Current	24.72	5.41		2065	21.71	0.73	50% GF	25.55	4.00
2065	21.28	3.82	2035	25.22	5.58		0%GF	22.09	0.73	2035	25.98	4.13
50% GF	21.86	4.42	100% GF	25.38	5.64		2100	22.61	1.22	2065	26.78	4.37
2100	21.18	4.53	2065	26.02	5.64		50% GF	25.55	4.00	2100	27.68	4.65
100% GF	25.38	5.64	2100	26.92	5.64		100% GF	29.01	5.05	100% GF	29.01	5.05

B

Grassholm Chicks (>50% black)							Malgas Chicks (>50% black)					
	Mean			Max				Mean			Max	
	Amb	EWL		Amb	EWL			Amb	EWL		Amb	EWL
0% GF	19.53	0.89	0% GF	19.53	0.89		Current	20.41	0.80	0% GF	22.62	0.80
Current	19.98	1.24	50% GF	24.52	4.84		2035	20.91	0.80	Current	25.48	4.00
2035	20.48	1.64	Current	24.72	4.89		2065	21.71	0.80	50% GF	25.79	4.35
2065	21.28	2.28	2035	25.22	5.03		2100	22.61	0.80	2035	25.98	4.42
2100	22.18	2.99	2065	26.02	5.24		0% GF	22.62	0.80	2065	26.78	4.73
50% GF	24.52	4.84	2100	26.92	5.48		50% GF	25.79	4.35	2100	27.68	5.07
100% GF	29.50	6.17	100% GF	29.50	6.17		100% GF	28.96	5.56	100% GF	28.96	5.56

By 2100, EWL for chicks (black) at Grassholm, under projections based on mean temperatures, will remain at 0.91% and increase to 5.55% hour^{-1} based upon maximum temperature projections. At Malgas, under mean projections chicks (black) will remain at their basal rate of 0.82% EWL, increasing to 5.13% based upon maximum temperature projections (Table 6A). At Grassholm, EWL will remain at the basal rate of 0.89% for adults, based on mean temperatures, increasing under maximum projections to 3.22%. For adults at Malgas, EWL will

remain at 0.81% under mean projections and increase to 4.67% under maximum projections (Table 6B).

Table 6. EWL hour^{-1} as a percentage of daily-ingested water for chicks (downy) **(A)** and chicks (>50% black) **(B)** across both sites, at ambient temperatures for gular fluttering threshold (0% GF), intermittent gular fluttering (50% GF) and heat stress (100% GF). Values are shown for current mean and maximum ambient temperatures and under climate change projections.

A

Grassholm Chicks (black)							Malgas Chicks (black)					
	Mean			Max				Mean			Max	
	Amb	EWL		Amb	EWL			Amb	EWL		Amb	EWL
Current	19.98	0.91	0% GF	22.50	0.91		Current	20.41	0.82	0% GF	23.77	0.82
2035	20.48	0.91	Current	24.72	3.85		2035	20.91	0.82	Current	25.48	3.31
2065	21.28	0.91	2035	25.22	4.51		2065	21.71	0.82	2035	25.98	4.02
2100	22.18	0.91	50% GF	25.53	4.93		2100	22.61	0.82	50% GF	26.28	4.45
0% GF	22.50	0.91	2065	26.02	5.15		0% GF	23.77	0.82	2065	26.78	4.70
50% GF	25.53	4.93	2100	26.92	5.55		50% GF	26.28	4.45	2100	27.68	5.13
100% GF	28.57	6.28	100% GF	28.57	6.28		100% GF	28.81	5.68	100% GF	28.81	5.68

B

Grassholm Adults							Malgas Adults					
	Mean			Max				Mean			Max	
	Amb	EWL		Amb	EWL			Amb	EWL		Amb	EWL
Current	19.98	0.89	Current	24.72	0.89		Current	20.41	0.81	0% GF	24.94	0.81
2035	20.48	0.89	2035	25.22	0.89		2035	20.91	0.81	Current	25.48	1.68
2065	21.28	0.89	0% GF	25.38	0.89		2065	21.71	0.81	2035	25.98	2.49
2100	22.18	0.89	2065	26.02	1.86		2100	22.61	0.81	2065	26.78	3.79
0% GF	25.38	0.89	2100	26.92	3.22		0% GF	24.94	0.81	50% GF	27.13	4.37
50% GF	27.96	4.80	50% GF	27.96	4.80		50% GF	27.13	4.37	2100	27.68	4.67
100% GF	30.55	6.11	100% GF	30.55	6.11		100% GF	29.33	5.57	100% GF	29.33	5.57

Discussion

Here I quantified the gular fluttering response of two species of seabird to variations in temperature and humidity, as well as how this varies with age. I then place this in the context of water loss and make predictions about how this might alter in the face of climate predictions. I discuss the implications of these findings for understanding the direct effects of increased temperature on birds and how this might vary between species.

Temperature models

Operative temperature was greater for black orbs than white orbs at the same ambient temperature, highlighting potential greater heat loads for birds with darker plumage, as demonstrated in previous studies (e.g. Hochscheid *et al.* 2002). These birds will likely show increased sensitivity to the direct effects of climate change.

Thermoregulatory behaviours

Birds exhibit a range of thermoregulatory behaviours such as changing body position to increase convective heat loss (Brown & Downs, 2003) and drooping of wings to increase air circulation (Bartholomew, 1966). However, gular fluttering is the key indicator of heat stress. Here the observation that birds were rarely seen to have both their head up and beak open without gular fluttering could permit colony wide monitoring of heat stress by counting the numbers of birds with their heads up and beaks open. However, caution should be taken if implementing these methods as birds often have their heads lifted when scanning the colony/sky, exhibiting skypointing behaviours (an elaborate social behaviour; Nelson, 1965) and when begging for food (chicks) (Nelson, 2010).

Gular fluttering in relation to temperature and humidity

Simple linear regressions revealed a significant positive relationship between ambient temperature and the proportion of time spent gular fluttering. This suggests that as temperatures increase, increased investment in thermoregulation is required to maintain thermal homeostasis. This in turn may lead to reduced investment in growth, reproduction and/or foraging activities (McNab, 2002). In this study, gular fluttering proportion was not related to humidity however, under climate change models (IPCC, 2013), predicted increases in future relative air humidity may reduce gular fluttering efficiency, as EWL is higher at low relative humidity when the gradient between the bird and the surrounding environment is greatest. Subsequently, future increases in relative humidity may force birds to gular fluttering for longer periods of time or invest more time in alternative methods of thermoregulation.

Gular fluttering models

The best gular fluttering model included all of the variables assumed to be important for gular fluttering, namely; ambient temperature, age category of bird, study site and humidity. Age and site were identified as important variables that explained gular fluttering proportion, enabling threshold predictions. The best model only explained 34.23% of the deviance, a relatively low goodness of fit. However, there are a number of likely reasons for this. Firstly, there is no account for wind speed. Exposure to prevailing winds can increase heat loss via convection (Whittow, 1986), with birds shifting body positions away from the grounds boundary layer of heat as heat loads increase (Downs & Ward, 1997) and increasing their use of behaviours that augment convective heat loss (Bartholomew & Dawson, 1979), reducing the need for evaporative cooling by

gular fluttering. Conversely, gular fluttering proportion may increase when temperatures are high and wind speeds are low. The orientation of the birds may also impact their thermoregulatory ability and gular fluttering efficiency. Under high heat loads nesting masked boobies *Sula dactylatra* orient themselves away from the sun, shielding vascular and heavily pigmented areas to enhance evaporative heat loss from the gular region and conductive heat loss from the feet through shading (Bartholomew, 1966). This orientation behaviour should also be expected for gannets and may be associated with age due to the varying proportions of dark feathers and the concomitant negative heat load effects (Hochscheid *et al.* 2002). We can further deduce that chicks (>50% black) and chicks (black) are unlikely to yield any thermoregulatory benefits from changing their orientation, due to their dark plumage. Heat load is likely to vary considerably at nest locations across the colony based upon differences in exposure to environmental conditions. Finally, whilst a broad range of gular fluttering proportions were recorded in this study, the loss of information due to the binary transformation of gular fluttering proportions to account for data distribution may have resulted the low percentage of deviance explained by the models presented here.

Gular fluttering thresholds

I found that gular fluttering onset and heat stress threshold temperatures varied by age and species, but the broad pattern of gular fluttering proportion increasing with ambient temperature was consistent across the study as expected.

At both colonies, the youngest birds - chicks (downy), initiated gular fluttering at the lowest ambient temperatures. Whilst we would expect larger birds to begin evaporative cooling at lower temperatures due to their relative surface area to volume ratios (Dawson, 1982; Schmidt-Neilsen, 1984), they may be better able to thermoregulate or have thermally advantageous plumage, minimising heat load (Hochscheid *et al.* 2002). Gular fluttering at lower temperatures may convey a long-term thermoregulatory advantage to chicks, allowing them to slow the rate of increase in the proportion of time spent gular fluttering as temperatures rise, minimising EWL over time. Gular fluttering onset temperature is lower at Grassholm than at Malgas for chicks (downy), chicks (>50% black) and chicks (black). This lower threshold for birds that cannot leave the colony may be indicative of thermal adaptation to the lower ambient temperatures at this site. As the greatest temperature increases are predicted to occur at higher latitudes (IPCC, 2013), these birds may be at an increased risk from the direct effects of increasing temperatures.

Heat stress (100% gular fluttering) occurs at lower temperatures for chicks (downy) and chicks (black) at Grassholm compared to Malgas but at higher temperatures for chicks (>50% black) and adult birds. At Grassholm, chicks (downy) become heat stressed at the lowest temperature, whilst at Malgas, chicks (black) become heat stressed at the lowest temperature. At both sites, adults have the highest heat stress temperature thresholds. Broadly, the rates of increase between onset and stress were steeper for birds at Malgas than Grassholm across all age categories. At both sites, the rates of increase were shallowest for chicks, with the steepest rate of increase in adult birds. At Malgas the rates of increase between the two thresholds were steeper for all ages,

likely due to the smaller size of birds at this site. A degree of caution must be taken when drawing conclusions from threshold values. Only the start and stressed thresholds for chicks (downy) and the start thresholds for the chicks (>50% black) did not have overlapping 95% confidence intervals. Whilst not statistically significant, the differences seen are biologically relevant and a lack of statistical significance may be due to sample size variation. Further testing should be carried out across a greater spatial and temporal scale to validate the results seen here.

Modelling evaporative water loss and climate change

The findings in this study suggest that EWL as a result of gular fluttering is greatest for chicks (black) and lowest for chicks (downy). Furthermore rates of EWL are greater for all age categories at Grassholm compared to Malgas. The differences seen between age categories and species can be attributed to variations in mass and the larger size of northern gannets. Chicks (black) are likely to be at risk of dehydration due to their high EWL rates, however chicks (downy) may be at greatest risk due to their low gular fluttering onset thresholds. By 2100 mean temperature projections show an increase in gular fluttering proportion for chicks (downy) and chicks (>50% black) at Grassholm and for chicks (downy) at Malgas. Maximum temperature projections show that gular fluttering proportion and EWL will increase for all age categories at both sites. At Grassholm, the three age categories of bird confined to the nest will show the biggest increases in the proportion of time spent gular fluttering and EWL. Most notably gular fluttering proportion for chicks (downy) at Grassholm will increase to 1 (constant), with EWL increasing to the maximum rate of 5.64% $hour^{-1}$.

Most birds can lose between 30 to 40% of their body weight in water before dying (Maclean, 2013). Therefore, although dehydration and death could occur in a single day, it is more likely that the effects of multiple days of water loss could accumulate and have long-term impacts on the individual. High levels of dehydration could lead to the deaths of birds constrained to the nest, whose only source of water is via ingested food, whilst adult birds that remain at the nest may well be forced to abandon in favour of future survival (Oswald *et al.* 2008; Sherley *et al.* 2012a). This will be particularly important for both northern and cape gannets as both species make relatively long foraging trips; Grassholm - 25.14±17.04 hours (Votier *et al.* 2010), Malgas - 29.73±11.1 hours (Pichegru *et al.* 2007). An increase in nest abandonment would likely have serious reproductive consequences as personal observations revealed high levels of nest predation by great black-backed gull *Larus marinus* at Grassholm and by kelp gulls *Larus dominicanus* and great white pelicans *Pelecanus onocrotalus* at Malgas. In response to increasing temperatures a gradual shift in both northern and cape gannet breeding phenology towards cooler months and an earlier onset of breeding may occur. We may also see a poleward spatial migration to nesting sites at cooler latitudes (Thomas *et al.* 2004; Fuller *et al.* 2010). These changes in breeding activity could permit greater population survival under future climate change scenarios. However, if the current timing of breeding activity for northern and cape gannets is linked to the availability of resources such as prey or nest sites then any changes to their breeding activity may result in poorer diets due to reduced prey availability or increased competition for resources such as nest sites. These in turn could have major

consequences for fledgling fitness, survival and long-term population recruitment.

Whilst the findings here indicate that increasing temperatures will have the greatest direct effect on northern gannets and the youngest age categories, projections are likely to be very conservative. Predictive modelling temperatures are based upon global averages, however increases over land are expected to be greater than those over water (IPCC, 2013). Furthermore, the frequency and duration of extreme high temperature events are also predicted to increase, with significant implications for seabird populations if they occur during the breeding season (IPCC, 2013). It should also be noted that in addition to increases in air temperature, current climate change models predict an increase in humidity and annual mean precipitation across high latitudes and the equatorial Pacific, whilst for many mid-latitude and subtropical dry regions, mean precipitation will likely decrease (IPCC, 2014). Whilst increased rainfall could aid the thermoregulation of nesting birds and increase the availability of water resources, minimising water loss, high levels of precipitation can also be costly (see Newell *et al.* 2015). Furthermore as mentioned above, increases in relative humidity could inhibit the efficacy of thermoregulation via evaporative cooling. Consequently, understanding the synergistic impacts of increasing temperatures, increasing humidity and regional changes in precipitation is essential when estimating rates of water loss and the costs of thermoregulation.

Finally, models for evaporative water loss were developed from previous studies (Hochscheid *et al.* 2002, Campbell, 2014; Dufour, 2015) and did not account for variation around estimates of FMR and DFI. Values were based

upon water intake solely from food, yet adult birds can drink water outside of this, resulting in potential inaccuracy with the values for the volume of water actually absorbed. Additionally, the actual water content of the prey species may vary based upon it fat content.

Further studies

Studies should capture footage across the breeding season, equally for all age categories, to maximise the potential range of temperatures that behavioural data is captured over. Studies should also record wet-bulb temperature to account for the reduced efficiency of evaporative cooling when relative humidity is higher. This may give a more accurate measure of gular fluttering and associated water loss (Budd, 2008). The inclusion of other thermoregulatory behaviours into models may help better explain deviance and identify the thermoregulatory processes used to lose heat as temperatures and heat loads increase. Recording the orientation of birds relative to the position of the sun may also provide insight into thermoregulatory ability, gular fluttering efficiency and net solar radiation gain by highlighting the occurrence of shielding behaviours to facilitate evaporative water loss and minimise heat gain (Bartholomew, 1966; Lustick, Battersby & Kelty, 1978).

Collecting data from multiple colonies will help validate the results presented here, providing insight into thermal vulnerability of individual colonies whilst data collected along a latitudinal gradient may highlight thermal adaptation within species. Gular fluttering onset may occur at lower ambient temperatures for birds breeding at higher latitudes. If this is the case then the direct impacts of increasing temperatures will likely vary across species based upon geographic

distribution of breeding colonies. Birds at lower latitudes may be closer to their thermal limits and critical air temperatures (Oswald & Arnold, 2012), whilst those at high latitudes may be vulnerable to heat stress due to morphological adaptations that minimise heat loss (Nudds & Oswald, 2007). Knowledge of the maximum temperatures that birds can cope with before abandoning their nests will also help determine future population survival. Studies may also wish to record morphological variations such as gular stripe length. Whilst gular stripe length has been shown to have a role in sex differentiation for cape gannets (Rishworth *et al.* 2014), it may also play a key role thermoregulatory role as an adaptation to aid heat loss. Personal observations found a greater length in gular stripe for cape gannets than northern gannets. This may be in response to the environmental differences between the study sites.

Finally, here I demonstrate the short-term impact of increasing temperatures for breeding birds but understanding the long-term impacts of heat stress is essential. Nestling development and post-fledging survival probability can be negatively affected by exposure to high temperatures (Rodriguez & Barba, 2016). High temperatures can cause oxidative stress and production of heat shock proteins, negatively affecting growth (Moreno *et al.* 2002) whilst hyperthermia can lower food intake, reducing growth rate, muscle development and delayed fledging (Cunningham *et al.* 2013a). Hyperthermia also increases energetic demands, leading to weight loss, acute dehydration and ultimately death (Catry *et al.* 2015). It is therefore important to establish projects which monitor the long-term impacts of increasing temperatures on individuals and populations. However, teasing apart the long-term individual impacts of the direct and indirect effects of climate change will be a considerable challenge.

Conclusion

This study found that gular fluttering correlates with temperature and that onset varied with age - the youngest birds starting to gular flutter at the lowest temperatures. Moreover, northern gannets began gular fluttering at lower temperatures for all age categories compared with Cape gannets, which may be a thermal adaptation to breeding at higher latitudes. EWL increased with gular fluttering and was higher for all age categories in northern compared to Cape gannets. Large black chicks had the highest rates of EWL, highlighting their sensitivity to dehydration. However while downy chicks had the lowest rates of EWL, they may be more prone to heat stress because of their low gular fluttering onset threshold. The effects of heat and evaporative stress are predicted to increase under climate change scenarios, with northern gannets particularly impacted. By 2100 downy chicks of northern gannets will have a maximum rate of EWL of 5.64% $hour^{-1}$, losing 28% of their daily-ingested water every 5 hours. Both gannet species may be able to buffer these effects behaviourally, although these may come at a cost. For chicks, long-term exposure to high heat loads may result in dehydration or death. For adults, increased temperatures will likely result in nest abandonment, in favour of future breeding success. This could ultimately prompt changes in breeding phenology or movement to more thermally favourable nesting sites, if such alternative locations exist. It is clear from the findings presented here that the direct effects of increasing temperatures have the potential to impact species fitness and survival. Further empirical research is essential to help enhance current understanding of the direct effects of climate change on birds, both in the short and long term. The findings from these studies should then be considered

alongside the indirect effects of increasing temperatures in future species distribution and survival models.

GENERAL DISCUSSION

Rising temperatures represent a major threat to avian populations globally and throughout the course of this century are likely to become an increasingly significant driver of behavioural, phenological and demographic changes, in addition to shifts in species geographic ranges and distributions (Pearce-Higgins & Green, 2014). Increasing temperatures can influence both individuals and populations through the direct effects on vital rates and via indirect effects as a result of temperature increases altering resource availability, such as prey abundance. The effects of these indirect effects can often be lagged and individually can be difficult to tease apart.

In recent years there has been a greater focus on the indirect effects of increasing temperatures on birds, as these have been perceived to be of greater importance due to the thermoregulatory adaptations that birds possess to withstand the challenges posed by environmental variation (Ockenden *et al.* 2014). However in chapter 1 I demonstrated that a diverse range of taxa are experiencing a wide array of direct effects, despite their physiological and behavioural adaptations. Birds found in marine, desert and tropical environments are likely to be the most vulnerable to increasing temperatures and resultant heat stress. For marine birds this is because of the thermoregulatory constraints of nesting on land and foraging at sea that they face (Oswald & Arnold, 2012), whilst desert and tropical birds are likely close to their thermal limits and at considerable risk of heat stress and mass mortality (McKechnie & Wolf, 2010; Albright *et al.* 2017). Furthermore for some species closer to the poles the evolutionarily advantageous adaptations that protect

against low environmental temperatures may no longer convey that evolutionary advantage and exacerbate the direct effects of rising temperatures and could have serious negative impacts on population recruitment (See Oswald *et al.* 2008). Whilst mass mortality events and reduced rates of survival appear to be the most immediately severe direct effects of increasing temperatures, the lack of information detailing the long-term effects at an individual and population level should also be a considerable cause for concern. These findings illustrate the necessity of long-term studies monitoring the direct effects of increasing temperatures on birds and highlight the importance of detailed knowledge of species-specific patterns of thermoregulation. Spatial variation in studies of the same species may also identify patterns of thermal adaptation within species and highlight variation in individual species responses to increasing temperatures. The results of these studies can then be incorporated into future species survival models and will inform critical management decisions and the implementation of conservation initiatives aimed at minimising the effects of climate change on birds.

In chapter 2 the use of remote sensing technologies, in conjunction with environmental records, allowed for examination of the differences in thermoregulatory behavioural responses of two closely related seabird species, in response to variation in ambient temperatures. Furthermore these patterns of behavioural response could be examined between distinct life history stages. This is turn led to estimations of the costs behavioural thermoregulation across age and species and predictions of the effects and costs that increasing temperatures due to climate change will have. The findings presented here show that the youngest birds are at greatest risk from increasing temperatures

and that the northern gannets appear to exhibit a degree of thermal adaptation to their higher latitude habitat, with the onset of thermoregulation occurring at lower ambient temperatures than for cape gannets. As ambient temperatures continue to increase the initial responses of both species will comprise of increased time spent exhibiting thermoregulatory behaviours. Long-term effects due to a continued increase in temperatures will likely include the mortality of young chicks and nest abandonment by adult birds resulting in reduced population recruitment. In some cases movement to more thermally favourable nesting sites or a temporal shift in breeding period may also occur.

This study utilised current research, novel research techniques and sound ecological knowledge of species patterns of thermoregulation to identify the direct effects of increasing temperatures on birds and to investigate the behavioural responses and costs of ambient temperature increases in two closely related seabird species. Whilst the results presented here should be considered carefully, the research highlights the importance of understanding the direct effects of increasing temperatures on birds and the incorporation of these effects into future models of species distribution and survival based upon climate change. This research also presents a solid foundation and methodology for future remote monitoring studies on the responses of birds to changing temperature at a species-specific level.

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APPENDICES

Appendix 1: Filming dates and durations for northern gannets at Grassholm Island (2016) and cape gannets at Malgas Island (2017).

Date	Site	Filming Times
21/07/16	Grassholm	12:00-19:00
22/07/16	Grassholm	11:00-18:00
23/07/16	Grassholm	10:00-19:00
05/08/16	Grassholm	09:00-19:00
06/08/16	Grassholm	08:45-15:00
09/01/17	Malgas	08:00-18:00
10/01/17	Malgas	06:00-19:00
11/01/17	Malgas	06:00-19:00
12/01/17	Malgas	07:00-19:00
13/01/17	Malgas	07:00-18:00
14/01/17	Malgas	07:00-20:00
15/01/17	Malgas	07:00-19:00

Appendix 2: Painted copper orbs to measure operative temperatures

Appendix 3: Stevenson's screen to record ambient temperature and humidity

Appendix 4: Camera set up for capturing behavioural data of northern gannets (left)) and cape gannets (right).

Appendix 5. Theoretical values of evaporative water loss at when gular fluttering 0% of the time, 50% of the time and 100% for each age category across both species. FMR: Field metabolic Rate, DFI: Daily Food Intake and EWL: Evaporative Water Loss.

Age Category	**Chicks (downy)**		**Chicks (>50% black)**		**Chicks (black)**		**Adult**	
Site	G	M	G	M	G	M	G	M
Mass (g)	1600	1200	3200	2500	3700	3000	3000	2600
FMR (kJ/d)	2409.09	1875.13	4406.06	3553.61	4999.98	4165.21	4165.21	3677.11
DFI	445.51	373.44	814.81	707.72	924.64	829.52	770.27	732.31
Ingested Water	356.41	298.75	651.84	566.17	739.71	663.62	616.21	585.85
EWL 0% GF	0.82	0.73	0.89	0.80	0.91	0.82	0.89	0.81
EWL 50% GF	4.42	4.00	4.84	4.35	4.93	4.45	4.80	4.37
EWL 100% GF	5.64	5.05	6.17	5.56	6.28	5.68	6.11	5.57